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Brit. J. Ophthal. (1957) **41**, 65.

COMMUNICATIONS

ACTION OF DIAMOX AND AMMONIUM CHLORIDE ON FORMATION OF AQUEOUS HUMOUR*

BY

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THE investigation was undertaken to establish the cause of the decrease in intra-ocular pressure induced in normal rabbits by 2-acetylamino-1, 3, 4 thiadiazole 5-sulphonamide†, an inhibitor of the enzyme carbonic anhydrase. At the same time, it was hoped that a comparison of these results with the known ability of Diamox to reduce the intra-ocular pressure in glaucomatous patients for extensive periods would help in assessing whether the intra-ocular dynamics of normal and pathological eyes are affected in a similar manner and to a comparable extent.

The lowering of the intra-ocular pressure by Diamox has led to the suggestion that the enzyme carbonic anhydrase is essential to the formation of the aqueous humour, and this idea has found expression in the hypothesis that the reduction of the intra-ocular pressure by Diamox is due to an inhibition of secretion across the blood-aqueous barrier (Becker, 1955a; Friedenwald, 1955a, b). This view had its origin in the studies of Friedenwald and Stiehler (1938) on the transfer of acidic and basic dyes across the epithelial layers of the ciliary processes. Friedenwald (1949) suggested that hydroxyl ions were secreted into the aqueous humour, and that an excess of bicarbonate was produced from the reaction of the hydroxyl ions with carbonic acid. This view was supported by Kinsey (1950), who observed in rabbits that the concentrations of hydroxyl and bicarbonate ions in the aqueous humour exceeded those in the plasma. The participation of carbonic anhydrase in this secretory activity was supported by the reports of the presence of the enzyme in extracts of the iris and the ciliary processes (Wistrand, 1951; Kauth and Sommer, 1953; Green, Capper, Bocher, and Leopold, 1954; Gloster and Perkins, 1955a).

Experiments designed to test either this hypothesis or alternative explanations of the action of Diamox in reducing the intra-ocular pressure have been reported by several investigators (Becker, 1955a, b, 1956; Becker and Constant, 1955; Green, Bocher, and Leopold, 1955; Langham and Lee, 1955a, b, c). There is general agreement that the reduction in the intra-ocular pressure is accompanied by a decreased rate of formation of aqueous humour, but the means by which this is brought about is still unresolved.

* Received for publication November 13, 1956.

† Diamox, Lederle Laboratories.

Becker (1955a, b, 1956) and Becker and Constant (1955) reported that their experimental results were consistent with the view that Diamox lowers the intra-ocular pressure by directly inhibiting the secretion of aqueous humour and in particular the transfer of excess bicarbonate into the aqueous humour. However, evidence that the ratio of the concentrations of bicarbonate between the aqueous humour and the blood was unchanged in animals given Diamox, in spite of the absolute decrease in the concentrations, led Langham and Lee (1955a, b) to question whether the production of excess bicarbonate in the eye in fact depends on a secretory mechanism involving carbonic anhydrase.

In the present study three aspects of this problem have been considered. First, the *p*H and the distribution of bicarbonate ions between the blood and the aqueous humour before and after the administration of Diamox have been determined. In particular, attention has been focused on possible differences between the composition of the fluids in transient and steady-state conditions. This was undertaken in view of the rapid decrease in the intra-ocular pressure that takes place immediately after giving Diamox (Becker, 1955a; Gloster and Perkins, 1955). Similar studies were made on animals with the renal arteries tied off and on animals given ammonium chloride in view of the interesting observations of Becker (1955a) that neither the administration of ammonium chloride nor nephrectomy made animals unresponsive to Diamox. Technically, this problem involved measurements of *p*H by the glass electrode technique and the analysis of total carbon dioxide in small volumes of fluid. Previously published papers relevant to this question include those of Kinsey (1950), Green, Bocher, and Leopold (1955), and Becker (1955). Kinsey (1950) measured the *p*H of the aqueous humour and the plasma by a dye technique, and the total carbon dioxide by the manometric technique of van Slyke (1926), and reported that the *p*H and the total concentration of carbon dioxide in the aqueous humour exceeded that in the plasma. Green and others (1955) reported that Diamox did not affect the concentration of total carbon dioxide in either the aqueous humour or the blood, and Becker (1955b) reported that Diamox reduced the concentration of total carbon dioxide in the aqueous humour but not in the blood. It is not at all evident from these last two papers why there should be a discrepancy between the results, but it is hoped that the more detailed approach used in the present study has avoided the errors inherent in the techniques used in previous studies. In particular, care has been taken to analyse whole arterial blood rather than plasma derived from a mixed arterial-venous sample obtained by cardiac punctures.

The second aspect of the problem concerns the effect of Diamox and ammonium chloride on the rate of formation of aqueous humour. For this purpose, fluorescein has been used as the test substance, and the concentration of fluorescein in the anterior chamber has been continuously recorded by a technique described in an earlier paper (Langham and Wybar, 1954).

The use of fluorescein to measure changes in the rate of formation of the aqueous humour by observing the time taken for the dye to pass through the posterior chamber was first suggested by Linnér and Friedenwald (1954). Since then the method has been employed in several studies (Becker, 1955c; Kornblueth and Linnér, 1955; Langham and Lee, 1955a, b). That fluorescein may be used in this way has found confirmation in a separate study (Langham and Wood, 1956).

The third aspect deals with the changes in the intra-ocular pressure in animals given Diamox or ammonium chloride during the period leading to a new condition of steady-state. This has assumed an increasing importance in view of observations that the effect of Diamox on the rate of formation of aqueous humour and distribution of bicarbonate ions across the blood-aqueous barrier was minimal in conditions of steady-state.

Methods

Adult rabbits of both sexes were used. They were fed Diet 18 pellets (Associated London Flour Millers), water *ad lib.*, and hay once a week. Samples of aqueous humour were taken after instilling a 1 per cent. solution of pantocaine into the conjunctival sac. All samples of aqueous humour were tested for the presence of protein by the addition of an equal volume of a solution of 8 per cent. (w/v) trichloroacetic acid, and the results were rejected if more than the normal faint clouding was observed. Blood samples were removed from the heat-dilated median artery of the ear. The Diamox for injections was prepared as a 10 per cent. solution of the sodium salt. Intravenous injections of fluorescein were made, using a 10 per cent. solution of the sodium salt.

The rate of accumulation of fluorescein in the anterior chamber was studied in rabbits anaesthetized with urethane (1·75 g./kg. body weight). Urethane in a 25 per cent. solution was slowly injected intravenously and 15 min. later 1·0 ml. of a 10 per cent. solution of sodium fluorescein was injected intravenously into the marginal ear vein within a period of 10 sec.

The ascorbic acid was dissolved in water, partially neutralized with a solution of sodium hydroxide to a *p*H of 6·5 to 7·2 and made up to an isotonic concentration. Raised concentrations of ascorbic acid in the plasma were maintained by an initial intravenous injection followed by hourly intramuscular injections for 6 hrs. A period of 6 hrs is sufficient to allow a steady-state to be reached in the aqueous humour to within a few per cent. (Bárány and Langham, 1955).

Analytical.—There are both theoretical and practical reasons for using whole blood rather than plasma to determine the *p*H and the concentration of total carbon dioxide in the plasma. Thus whole blood may be analysed immediately, and provided the haemoglobin and water content are known, the plasma values may be derived directly from the nomograms of van Slyke and Sendroy (1928). In contrast, to obtain plasma, the blood has first to be centrifuged, which involves difficulties in maintaining the composition and *p*H constant. Consequently, all determinations were made on freshly withdrawn samples of arterial blood. To enable the *p*H and the total carbon dioxide measurements to be made on the aqueous humour of each rabbit, samples from the two eyes were withdrawn into the same syringe and the pooled sample analysed immediately. In this way it was possible to take duplicate determinations of the *p*H and the total carbon dioxide on each sample. The alternative method of using the aqueous humour of one eye for both analyses was rejected in view of the larger errors involved in making analyses on volumes of less than 0·15 to 0·20 ml.

The *p*H of the blood and the aqueous humour was estimated using a Cambridge model *p*H meter with a micro glass electrode and cell. Dead space in the cell was reduced to approximately 0·75 ml., and the sample was introduced from a syringe directly into the chamber through a capillary tube connexion. The cell was immersed in a saturated solution of KCl which acted as a water bath as well as a junction to the calomel half-cell.

To obtain stable *p*H readings on the small samples of aqueous humour, it was found imperative to dry the micro-cell and prevent any mixing of the aqueous humour in the cell with the KCl in the connecting capillary. The glass electrode cannot, however, be dried, and it is important to wash the electrode with CO_2-free glass-distilled water of *p*H 7·0 in view of the limited buffering power of the aqueous humour. The temperature correction factor was estimated separately (see Results). In these conditions the *p*H of the blood and the aqueous humour was found to remain unchanged for at least 3 min. *p*H readings were taken within 1 to 2 min. of removing the sample from the animal. Standard buffers of known *p*H were run at the beginning and end of each run of readings.

The total CO_2 of the blood and the aqueous humour was determined by the manometric technique of van Slyke and Neill (1924). To derive the concentration of total CO_2 in the plasma, use was made of the nomogram compiled by van Slyke and Sendroy (1928); this relates *p*H to the oxygen capacity of the blood and gives the factor by which the CO_2 content of whole blood may be converted to plasma CO_2. For these calculations the oxygen capacity of rabbit blood was determined separately on a group of six animals, and a mean value of 19·1 ± 0·3 ml./100 ml. was recorded. The conversion factor changes less than 1 per cent. for a change in the oxygen capacity of from 19 to 20 vols per cent.

The concentrations of bicarbonate and CO_2 were calculated from the total CO_2 concentration and the blood *p*H by using the Henderson-Hasselbach equation, In this equation the *p*K for the bicarbonate system in blood was taken as 6·10 and in aqueous humour as 6·13. The latter figure is that given by Warburg (1922) for a protein-free bicarbonate solution containing 150 m. moles NaCl per kg. H_2O, which is an equivalent ionic concentration to the aqueous humour.

The CO_2 tension in the blood and the aqueous humour was calculated from the CO_2 concentration; $mM(H_2CO_3) = \frac{1{,}000\alpha CO_2}{22{\cdot}4} \times \frac{pCO_2}{760}$ where αCO_2 represents the ml. CO_2 dissolved per g. H_2O at 760 mm., and pCO_2 represents the tension in mm. Hg. The value of CO_2 substituted in the above equation was 0·533 (van Slyke, Sendroy, Hastings, and Neill, 1928); the equation simplifies to $mMH_2CO_3/kg.H_2O = 0{\cdot}0315pCO_2$.

The mean water content of the aqueous humour was taken as 99 per cent., and in a series of ten determinations the water content of whole blood was found to be 82·3 ± 0·30 per cent. Direct determination of the concentrations of total carbon dioxide in the plasma agreed with the values derived from the analysis of whole blood.

Ascorbic acid in samples of aqueous humour were analysed immediately after withdrawal by the procedure previously described (Bárány and Langham, 1955).

The concentration of sodium in the aqueous humour and the plasma was determined by the standard flame photometric technique by the procedure previously described (Langham and Taylor, 1956).

Measurement of the Intra-Ocular and Blood Pressures.—Rabbits were anaesthetized by the intraperitoneal injection of a 25 per cent. solution of urethane (1·75 g./kg.). The femoral artery on one side was cannulated for the blood pressure record. The intra-ocular pressure was measured by the technique of Greaves and Perkins (1952).

Measurement of the intra-ocular pressure in conscious animals was made by a tonometric technique using a standard Schiötz X tonometer. The animal was placed in a canvas bag tied around the neck to restrain undue movement and one drop of pantocaine (1 per cent.) was placed in each eye. This was washed off almost immediately with saline to prevent corneal drying. Two readings were recorded on each eye.

Results

Chemical Studies.—The *p*H of the blood and the aqueous humour were measured at room temperature and the values corrected to body temperature. Rosenthal (1948) determined the *p*H-temperature coefficient for whole rabbit blood and reported a value of 0·0147±0·00020 *p*H units per °C.; von Sallman and di Grandi (1946) reported the *p*H of the aqueous humour of 24 rabbits to be 7·53 at 36·5 C.° and the *p*H of the aqueous humour of ten rabbits to be 7·63 at 23° C. In the present study the *p*H-temperature coefficients of freshly-drawn arterial blood and of the aqueous humour of twelve conscious rabbits were determined. The mean value for blood was 0·0147±0·00020 *p*H units per °C. and for the aqueous humour it was 0·0064±0·00025 *p*H units per °C. Throughout the temperature range studied, namely 15 to 37·5° C., the temperature coefficients for blood and for aqueous humour were found to be constant and the *p*H effect fully reversible. In the following results the *p*H of the blood and the aqueous humour have been corrected by the use of these factors; the mean temperature of the aqueous humour has been taken as 36·5° C. (von Sallman and di Grandi, 1946).

The mean *p*H, CO_2 tension, and concentrations of total CO_2 and bicarbonate in the arterial plasma and aqueous humour of conscious rabbits are recorded in Table I. The concentrations of bicarbonate and hydroxyl ions in the aqueous humour exceeded those in the arterial plasma and are in general agreement with the observations of Kinsey (1950). In addition, the calculated value for the CO_2 tension in the aqueous humour agreed with the mean value of 35·1 mm. Hg reported by Pierce, Friedenwald, and Freeman (1933). These workers introduced a bubble of nitrogen into the anterior chamber of dogs, and analysed the CO_2 content in samples of the bubble after increasing time intervals. The observation that the CO_2 tension in the blood and the aqueous humour are approximately equal was to be expected in view of the relatively small molecular size, the lipoid solubility of carbon dioxide, and the ease with which lipoid-soluble molecules penetrate the blood aqueous barrier (Ross, 1951; Langham, 1955a).

The effect of Diamox on the composition of arterial blood and aqueous

TABLE I

COMPOSITION OF ARTERIAL PLASMA AND AQUEOUS HUMOUR OF NORMAL CONSCIOUS RABBITS

Plasma values calculated from analysis on arterial blood
Concentrations expressed in mM/kg.H_2O and CO_2 tension in mm.Hg

	Plasma	Aqueous Humour	Aq–Pl	Aq/Pl
*p*H	7·43±0·012 (12)	7·60±0·020 (12)	0·17±0·02	—
H^+	$3{\cdot}71\times10^{-8}$	$2{\cdot}51\times10^{-8}$	$-1{\cdot}20\times10^{-8}$	0·68
Total CO_2	24·2±1·05 (12)	31·6±1·19 (12)	7·4±0·96	1·31
HCO^-_3	23·1±1·07 (12)	30·5±1·20 (12)	7·4±0·90	1·32
pCO_2	35±1·2 (12)	35±1·2 (12)	0·0±0·8	1·00

humour was studied approximately 15 and 150 minutes after an intravenous injection. The time interval of 15 minutes was chosen in view of the known ability of Diamox given intravenously to cause a significant reduction in the intra-ocular pressure within this time (Gloster and Perkins, 1955b). The period of 150 minutes was based on preliminary experiments which showed that the fall in the *p*H and in the concentration of bicarbonate in the aqueous humour and the blood after the administration of Diamox approached to within 10–20 per cent. of the steady-state values in 1 to 2 hrs. Fig. 1 shows the concentration of total carbon dioxide in the arterial blood of individual rabbits given repeated doses of Diamox. It was found that the concentration of total carbon dioxide in the plasma decreased by approximately 6 mM/kg. H_2O in 30 to 60 minutes.

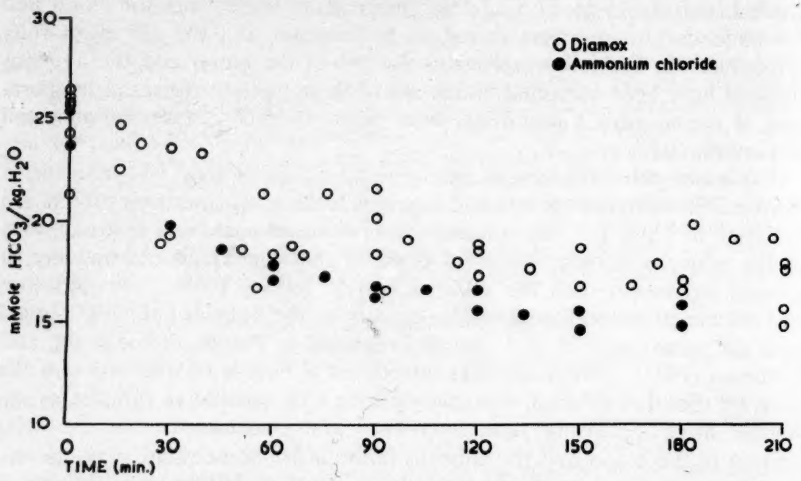

FIG. 1.—Concentration of bicarbonate in arterial plasma of conscious rabbits given Diamox intravenously (50 mg./kg. initially followed by 25 mg./kg. hrly) and ammonium chloride orally (270 mg./kg.).

The composition of the plasma and aqueous humour of conscious rabbits given an intravenous injection of Diamox 15 minutes previously are recorded in Tables II and III (opposite). The *p*H of the blood was significantly below that in the control series but the concentration of bicarbonate remained unchanged. The calculated value of pCO_2 in the blood indicated a significant increase. A similar elevation in the CO_2 tension and a fall in the *p*H of arterial blood in human subjects 30 to 60 minutes after the oral administration of Diamox in doses of 25 mg./kg. has been reported by Cranston, Sanderson, and Stapleton (1955). Further indications that Diamox can cause a temporary elevation of arterial CO_2 tension is to be seen in the experimental results in dogs of Maren, Wadsworth, Yale, and Alonso (1954).

The *p*H in the aqueous humour was also lower than in the control series

TABLE II
EFFECT OF DIAMOX (50 mg./kg. BODY WT.) ON COMPOSITION OF ARTERIAL PLASMA AND AQUEOUS HUMOUR
Samples were removed 15 min. after intravenous injection of Diamox

	Plasma	Aqueous Humour	Aq–Pl	Aq/Pl
*p*H	7·34±0·013 (12)	7·45±0·020 (12)	0·11±0·02 (7)	—
H^+	$4{\cdot}57\times10^{-8}$	$3{\cdot}55\times10^{-8}$	$-1{\cdot}02\times10^{-8}$	0·77
Total Co_2	25·3±0·58 (12)	26·0±0·50 (11)	0·70±0·55 (7)	1·03
HCO^-_3	23·9±0·63 (12)	24·8±0·51 (11)	0·80±0·47 (7)	1·03
pCO_2	44±1·8 (12)	38±1·9 (11)	−6±2·35 (7)	0·86

TABLE III
EFFECT OF 20 mg. DIAMOX/kg. BODY WT. ON COMPOSITION OF ARTERIAL PLASMA AND AQUEOUS HUMOUR OF CONSCIOUS RABBITS
Samples withdrawn 15 minutes after intravenous injection of Diamox

	Plasma	Aqueous Humour	Aq–Pl	Aq/Pl
*p*H	7·33±0·014 (9)	7·51±0·025 (9)	0·18±0·023	—
H^+	$4{\cdot}68\times10^{-8}$	$3{\cdot}10\times10^{-8}$	$-1{\cdot}58\times10^{-8}$	0·66
Total CO_2	26·2±1·01 (9)	28·5±1·60 (9)	2·3±1·30	1·09
HCO^-_3	24·7±1·02 (9)	27·4±1·57 (9)	2·3±1·77	1·11
pCO_2	46±2·5	35±1·3	−11±3·6	0·76

but it remained significantly above that in the blood. The pCO_2 was not significantly above that in the control series, but the differences in the values of the aqueous humour and blood of individual rabbits showed a greater scatter than in the control series. In general the pCO_2 in the blood was higher than in the aqueous humour. The concentration of bicarbonate, unlike that in the blood, was significantly lower than in the control series and closely approached that in the plasma. Thus, the excess concentration of bicarbonate in the aqueous humour of the two series of rabbits given Diamox dropped nearly to zero within 15 minutes.

The composition of the arterial blood and aqueous humour of conscious rabbits 2·5 hrs after an initial intravenous injection of Diamox are recorded in Tables IV and V (overleaf). Two series of experiments were made using different dosages of Diamox to see whether the effect varied appreciably with the amount of drug given. In the first series an initial injection of 20 mg./kg. followed by 10 mg./kg. at hourly intervals was administered, and in the second series the dosage was increased five times. In spite of this difference the response in the two series appeared to be very similar. The dosage of 100 mg./kg. is at least twenty times that which will induce an appreciable fall in the intra-ocular pressure of rabbits (Gloster and Perkins, 1955b). The *p*H of the blood was similar to that measured 15 minutes after giving Diamox, but the concentration of bicarbonate in the plasma showed a significant decrease. The pCO_2 was similar to that in normal rabbits and in this respect differed from the elevated values shown in Tables II and III. Thus the increase of the pCO_2 was only a transient effect seen in the period immediately after Diamox was given.

TABLE IV

EFFECT OF DIAMOX GIVEN INTRAVENOUSLY 2·5 HRS PREVIOUSLY TO CONSCIOUS RABBITS

Initial intravenous dose of 20 mg./kg. body wt. followed by 10 mg./kg. body wt. 1 and 2 hrs later

	Plasma	Aqueous Humour	Aq–Pl	Aq/Pl
*p*H	7·34±0·022 (8)	7·44±0·018 (8)	0·10±0·03	—
H^+	$4{\cdot}57\times10^{-8}$	$3{\cdot}63\times10^{-8}$	$-2{\cdot}31\times10^{-8}$	0·79
Total CO_2	19·2±0·88 (8)	25·3±0·95 (8)	6·1±0·30	1·32
HCO^-_3	18·2±0·87 (8)	24·2±0·92 (8)	6·0±0·26	1·33
pCO_2	34±1·3	35±1·7	1±2·1	1·03

TABLE V

EFFECT OF DIAMOX ON COMPOSITION OF ARTERIAL PLASMA AND AQUEOUS HUMOUR

Diamox 100 mg./kg. given intravenously followed by 50 mg./kg. after 60 and 120 minutes. Samples removed at 2·5 hrs

	Arterial Plasma	Aqueous Humour	Aq–Pl	Aq/Pl
*p*H	7·36±0·01 (12)	7·50±0·02 (11)	0·13±0·03	—
H^+	$4{\cdot}36\times10^{-8}$	$3{\cdot}16\times10^{-8}$	$-1{\cdot}20\times10^{-8}$	0·72
Total CO_2	17·6±0·50 (12)	23·1±0·30 (10)	5·4±0·46	1·31
HCO^-_3	16·7±0·50 (12)	22·2±0·30 (10)	5·5±0·38	1·33
pCO_2	29±1·1 (12)	29±1·2 (10)	0±1·9	1·0

The *p*H of the aqueous humour was similar to that observed 15 minutes after the administration of Diamox, and again exceeded that in the plasma. The concentration of bicarbonate in the aqueous humour also showed no change compared with the value after 15 minutes. However, owing to the decrease in the concentration of bicarbonate in the plasma, there was an excess concentration of bicarbonate in the aqueous humour similar to that in untreated animals.

The concentrations of total carbon dioxide in the aqueous humour and plasma after the administration of Diamox have been reported by Green, Bocher, and Leopold (1955) and by Becker (1955b). Unfortunately these authors' results do not agree with the present results. Green and others (1955) reported that Diamox caused no change in the concentrations of total CO_2 in the aqueous humour or plasma of rabbits. No measurements of *p*H were taken, but it is evident from their results that many of their animals were in varying stages of acidosis before the start of the experiments, for over half of the animals injected with Diamox had an initial concentration of total CO_2 in the plasma of less than 20 mM/l. This contrasts with the values of 24·2±1·05 (12) mM/kg. H_2O in the plasma of normal rabbits having a *p*H of 7·43±0·012 (12). Their method of analysis is also open to criticism (Grant, 1956). In an agreement with the present observation, Becker (1955b) found that Diamox decreased the concentration of bicarbonate in the aqueous humour. However, Becker also reported that Diamox

had no significant effect on the concentration of bicarbonate in the plasma. This is difficult to understand, in that it is widely recognized that Diamox, even in a very low dosage, will cause an excretion of bicarbonate into the urine and a consequent decrease in the concentration of bicarbonate in the blood. The explanation may, however, lie in the method of analysis. The samples of plasma analysed by Becker were taken from the heart and would therefore comprise a variable mixture of arterial and venous blood. In comparison, the present analyses were made immediately on samples of arterial whole blood freshly drawn from the heat-dilated median artery of the ear.

Effect of Diamox on the Composition of the Blood and the Aqueous Humour of Rabbits in which Kidney Function has been Eliminated.—In the first series of rabbits, the blood and aqueous humour of untreated animals were analysed 3 hours after the operation; in the second series 50 mg. Diamox/kg. was injected intravenously 30 minutes after the operation and samples of aqueous humour and arterial blood were taken 2·5 hours later. The results in the untreated animals are recorded in Table VI. The *p*H and the concentration of bicarbonate in the blood and aqueous humour were significantly below those in normal animals. The concentration of bicarbonate in the aqueous humour of individual rabbits was, however, significantly above that in the blood and similar in value to that in normal rabbits.

TABLE VI

COMPOSITION OF ARTERIAL PLASMA AND AQUEOUS HUMOUR OF ANIMALS 3 HRS AFTER LIGATION OF RENAL ARTERIES

	Plasma	Aqueous Humour	Aq–Pl	Aq/Pl
*p*H	7·29±0·02 (5)	7·39±0·02 (5)	0·10±0·02	—
H^+	$5{\cdot}13\times10^{-8}$	$4{\cdot}26\times10^{-8}$	$-1{\cdot}13\times10^{-8}$	0·83
Total CO_2	20·0±1·15 (5)	26·6±0·35 (5)	6·6±1·25	1·33
HCO_3	18·8±1·06 (5)	25·2±0·35 (5)	6·4±1·18	1·34
pCO_2	38±3·5	44±0·7	6±3·4	1·16

The effect of Diamox on the operated rabbits is recorded in Table VII. The *p*H of the blood and aqueous humour dropped below the mean values recorded in the untreated series, and calculation of the CO_2 tension showed this to increase to high values compared with the tensions in untreated

TABLE VII

EFFECT OF DIAMOX (50 mg./kg.) ON COMPOSITION OF ARTERIAL PLASMA AND AQUEOUS HUMOUR OF RABBITS 3 HRS AFTER LIGATION OF RENAL ARTERIES

Diamox given intravenously 30 min. after ligation of renal arteries

*p*H	7·10±0·05 (5)	7·25±0·03 (4)	0·17±0·06	—
H^+	$7{\cdot}94\pm10^{-8}$	$5{\cdot}62\times10^{-8}$	$-2{\cdot}32\times10^{-8}$	0·71
Total CO_2	25·1±1·9 (5)	29·4±2·0 (4)	5·2±1·0	1·22
HCO_3	22·8±1·8 (5)	27·4±2·0 (4)	6·0±0·83	1·28
pCO_2	73±4·1	64±1·5	−7±5·9	0·88

rabbits. The concentrations of bicarbonate in the blood and aqueous humour were higher than in the untreated animals. In individual animals, the concentration of bicarbonate in the aqueous humour again exceeded that in the arterial plasma by an amount similar to that seen in the control and the normal series of rabbits.

The condition of acidosis observed in these animals is at variance with the observations of Becker (1955b). He reported that the concentrations of total carbon dioxide in the plasma and the aqueous humour of rabbits nephrectomized under pentabarbital anaesthesia 18 to 24 hours previously did not differ from those in unoperated animals. In the present study the decision to use animals 3 rather than 24 hours after the operation was prompted by the desire to keep pathological changes to a minimum. Thus at 24 hours the blood pressure and the intra-ocular pressure are below normal and the blood-aqueous barrier abnormally permeable to fluorescein. In view of Becker's observations, however, samples of the aqueous humour and blood of animals nephrectomized 24 hours previously were also made. The results did not differ significantly from those recorded in Tables VI and VII. In three control rabbits the mean concentrations of total carbon dioxide in the plasma and in the aqueous humour were 17·5 and 24·5 mM/kg. H_2O respectively, and in the animals given Diamox (50 mg./kg. 2·5 hrs previously) the corresponding values were 16·5 and 21·0 mM/kg. H_2O.

Chemical analysis of the blood and aqueous humour of rabbits after the administration of ammonium chloride was made in order to help interpret the observations of Becker (1955a) that Diamox lowers the intra-ocular pressure of animals pre-treated with high doses of ammonium chloride by mouth. A similar amount of ammonium chloride (270 mg./kg.) to that used by Becker and recommended by Maren and Wadsworth (1954) to block the renal effect of Diamox, was given to conscious rabbits. Repeated analysis of the concentration of total CO_2 in the blood showed a fall in concentration to approximately 16 mM/kg. H_2O within 60 minutes and little further change in the following 2 hours.

The composition of the plasma and aqueous humour of conscious rabbits 2·5 hours after the oral administration of 270 mg. NH_4Cl/kg. are recorded in Table VIII. The concentration of bicarbonate in the aqueous humour of individual animals exceeded that in the arterial plasma, but the excess

TABLE VIII
ANIMALS GIVEN 270 mg. AMMONIUM CHLORIDE/kg. BY STOMACH TUBE 2·5 HRS EARLIER

	Plasma	Aqueous Humour	Aq–Pl	Aq/Pl
*p*H	7·14±0·02 (12)	7·29±0·02 (12)	0·15±0·29	—
H^+	$7{\cdot}25\times10^{-8}$	$5{\cdot}12\times10^{-8}$	$-2{\cdot}13\times10^{-8}$	0·70
Total CO_2	18·7±0·84 (12)	21·5±0·82 (12)	2·8±0·90	1·15
HCO_3	17·1±0·76 (12)	20·1±1·83 (12)	3·0±0·86	1·18
pCO_2	51±1·9	44±2·8	−7±2·9	0·86

was considerably less than in untreated animals. The *p*H and the concentrations of bicarbonate in the plasma and the aqueous humour were well below those in untreated animals, and the calculated value for the CO_2 tension exceeded that in normal animals. The increased acidity was probably caused mainly by the acid nature of ammonium chloride and differs in this respect from the more prolonged action of ammonium chloride in producing a hyperchloraemia and a fully compensated type of metabolic acidosis (Haldane, 1921; Haldane, Linder, Hilton, and Fraser, 1928).

The composition of the plasma and aqueous humour of animals given ammonium chloride 270 mg./kg. by mouth followed by Diamox intravenously is recorded in Table IX. The concentration of bicarbonate in the aqueous humour exceeded that in the arterial plasma by an amount similar to that seen in the previous series of experiments; the *p*H of the blood and aqueous humour appeared to be slightly higher than in the previous series, and the bicarbonate concentrations were essentially unchanged. The calculated value for the CO_2 tension in the blood and aqueous humour showed them to be similar to those in normal rabbits.

TABLE IX

ANIMALS GIVEN 270 mg. AMMONIUM CHLORIDE/kg., 50 mg. DIAMOX/kg. BEING INJECTED INTRAVENOUSLY AFTER 1·5 HRS AND A FURTHER 25 mg./kg. AFTER 1 HR

Samples removed 3·5 hrs after administration of ammonium chloride

	Plasma	Aqueous Humour	Aq−Pl	Aq/Pl
*p*H	7·22±0·015 (12)	7·40±0·072 (12)	0·18±0·02	—
H^+	$6{\cdot}04\times10^{-8}$	$3{\cdot}98\times10^{-8}$	$-2{\cdot}06\times10^{-8}$	0·66
Total CO_2	17·5±0·85 (12)	20·3±0·84 (12)	2·8±0·93	1·16
HCO_3	16·3±0·81 (12)	19·3±0·82 (12)	3·0±0·92	1·18
pCO_2	38±1·6	32±0·9	−6±3·4	0·84

Effect of Diamox on the Concentration of Sodium in the Blood and Aqueous Humour.—In view of the observations that Diamox caused a decrease in the concentration of bicarbonate in the aqueous humour of approximately 6 mEq/kg. H_2O, studies were made to see if a corresponding decrease occurred in the concentration of the main cation, sodium. In ten control rabbits the mean concentration of sodium in the first eye was 135·6±0·99 mEq/kg. H_2O, and in the second eye, removed 15 min. later, it was 135·9±1·02 mEq/kg. H_2O. In the same series the mean difference in the concentration of sodium between the second and first eyes of individual rabbits was 1·0±0·21 mEq/kg. H_2O.

The effect of Diamox on the concentration of sodium in the aqueous humour and arterial plasma of four conscious rabbits given Diamox is recorded in Table X (overleaf). In two animals the concentration of sodium in the plasma and aqueous humour showed an increase, while in the other two the changes were not significant.

TABLE X

CONCENTRATION OF SODIUM IN PLASMA AND AQUEOUS HUMOUR OF CONSCIOUS RABBITS BEFORE (Pl_1 AND Aq_1) AND 20 MINUTES AFTER (Pl_2 AND Aq_2) INTRAVENOUS INJECTION OF DIAMOX (50 mg./kg.)

Results expressed as mEq/kg. H_2O

Pl_1	Pl_2	Aq_1	Aq_2	Aq_1/Pl_1	Aq_2/Pl_2
151	155	136	139	0·89	0·90
144	145	137	136	0·95	0·94
149	151	136	134	0·91	0·89
151	156	139	144	0·92	0·92

In separate experiments the concentrations of sodium in the blood and aqueous humour of untreated rabbits were compared with analyses made on conscious rabbits given Diamox (50 mg./kg.) intravenously 2·5 hours previously. The results, summarized in Table XI, showed that there was no significant change in the concentration of sodium in the plasma or aqueous humour.

TABLE XI

EFFECT OF DIAMOX ON CONCENTRATION OF SODIUM IN ARTERIAL PLASMA AND AQUEOUS HUMOUR OF CONSCIOUS RABBITS

Series A represents a control group of rabbits.
Animals in Series B were given 50 mg. Diamox/kg. intravenously and a further 25 mg./kg. 1 and 2 hrs later.

Samples withdrawn 2·5 hrs after initial injection

Series	Plasma	Right Aqueous	Left Aqueous	Aq/Pl
A	143·6±1·64 (10)	135·6±0·99 (10)	135·9±1·02 (10)	0·945±0·01 (10)
B	144·9±1·30 (10)	136·1±1·18 (10)	136·8±1·33 (10)	0·945±0·01 (10)

Effect of Diamox and Ammonium Chloride on the Transfer of Ascorbic Acid across the Blood-Aqueous Barrier.—In view of the observation that in certain respects ammonium chloride affects the formation and composition of the aqueous humour in a similar way to Diamox, it was considered of value to compare its action with that of Diamox on the accumulation in the aqueous humour of a compound known to be actively transported across the blood-aqueous barrier, namely, ascorbic acid. These results have been reported in a preliminary communication to the Physiological Society (Langham and Lee, 1955c).

In rabbits, guinea-pigs, and humans, it has been shown that a rise in the concentration of ascorbic acid in the plasma leads to an increase in the concentration in the aqueous humour, even though the raised plasma level may be considerably below that of the aqueous humour (Kinsey, 1947; Langham, 1950; Linnér, 1952; Bárány and Langham, 1955). In rabbits the secretion of ascorbic acid into the aqueous humour reaches a maximum at a plasma concentration of 2–3 mg./100 ml. and gives a mean concentra-

tion of 67·5±2·1 mg./100 ml. in the aqueous humour (Bárány and Langham, 1955).

The effect of Diamox and ammonium chloride on the secretion of ascorbic acid into the aqueous humour of conscious rabbits is recorded in Table XII. The concentration of ascorbic acid in the plasma of these animals was kept at a mean value of 5–10 mg./100 ml., which well exceeds the level of 2–3 mg./100 ml. required to ensure a maximal rate of transfer in normal rabbits. This was done to exclude a possible vasomotor action of Diamox in influencing the rate of secretion of ascorbic acid; thus the rate of transfer of ascorbic acid may be varied by a change in uveal blood flow (Linnér, 1952; Langham, 1955b). The aqueous humour from one eye was removed 3 hours after the initial injection of ascorbic acid. At this time the concentration of ascorbic acid in the aqueous humour had approached the maximum steady-state value of approximately 70 mg./100 ml. (Table XII). Then Diamox or ammonium chloride was given to the two experimental series of animals, and the aqueous humour in the second eye was removed 3 hours later. The results showed that the secretion of ascorbic acid had been maintained in these conditions. The analyses of Becker (1956) of the concentration of ascorbic acid in the aqueous humour of nephrectomized rabbits after giving Diamox are similar to those recorded here.

TABLE XII

EFFECT OF DIAMOX (50 mg./kg. FOLLOWED BY 25 mg./kg. AT HOURLY INTERVALS) AND AMMONIUM CHLORIDE (270 mg./kg.) ON CONCENTRATION OF ASCORBIC ACID (mg./100 ml.) IN AQUEOUS HUMOUR OF CONSCIOUS RABBITS

Ammonium chloride (orally) and Diamox (intravenously) were given after removal of Aq_1. All animals were given an initial intravenous injection of a solution of sodium ascorbate (*p*H 7·0), followed by intramuscular injections hourly to maintain the concentration of ascorbic acid in the plasma at 5–10 mg./100 ml.

Series	Aq_1 (3 hrs)	Aq_2 (6 hrs)	Aq_2–Aq_1
Controls	62·5±2·6 (8)	69·9±3·2 (8)	7·4±0·9
NH_4Cl	61·3±2·4 (8)	76·0±2·6 (8)	13·7±1·5
Diamox	65·3±1·6 (8)	83·3±2·1 (8)	18·0±2·1

Effect of Diamox and Ammonium Chloride on Transfer of Fluorescein from the Bloodstream into the Anterior Chamber.—The concentrations of fluorescein in three areas of the anterior chamber after an intravenous injection of a solution of sodium fluorescein are shown in Fig. 2 (overleaf). It took about 4 minutes for fluorescein to pass in a significant amount into the anterior chamber but then the concentration in front of the lens started to increase rapidly. The concentrations of fluorescein in the central and corneal areas of the anterior chamber lagged behind that in the lens area for approximately 30 minutes. In the period of approximately 4 minutes before the dye was recorded in a significant concentration in the anterior chamber, the aqueous humour, illuminated by the focal vertical beam of light from the slit lamp, changed from black to a faint greyish and then just perceptibly

green colour. This was, however, too little to record on the fluorophotometer and was probably due to the occasional streamline of fluorescein which passes through the pupil (see Friedenwald and Becker, 1955).

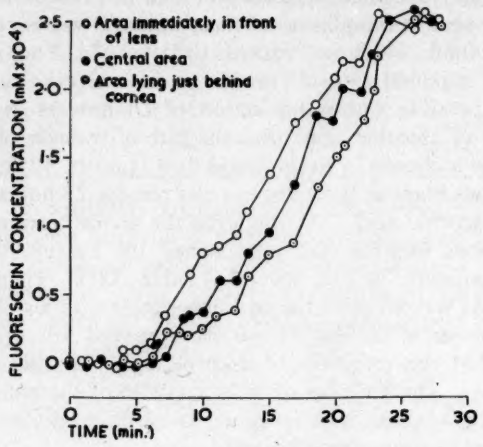

FIG. 2.—Concentration of fluorescein in three areas of aqueous humour after intravenous injection of 1·0 ml. of a 10 per cent. solution of sodium fluorescein to an anaesthetized rabbit.

In a series of ten anaesthetized rabbits the mean time taken for fluorescein to appear in the area of the anterior chamber immediately in front of the lens was 248±26 seconds. This value, which is based on the curve of accumulation, exceeds those reported by Linnér and Friedenwald (1954) and Kornblueth and Linnér (1955), who recorded the times at which a green colour was first perceptible at the pupillary margin.

The ability of Diamox to modify the appearance time of fluorescein was studied in rabbits 15, 60, 180, and 360 minutes after an intravenous injection. The results are recorded in Table XIII. At 15 and 60 minutes after the administration of Diamox, the appearance times were significantly above the mean value of the control series, but at 180 and 360 minutes the values did not exceed the controls, even though Diamox had been administered at hourly intervals.

TABLE XIII

TIME TAKEN FOR FLUORESCEIN TO PASS FROM THE BLOODSTREAM INTO THE AREA OF THE ANTERIOR CHAMBER IMMEDIATELY IN FRONT OF THE LENS

Diamox given intravenously in the stated dosage and repeated hourly at half this dosage

Series		Time (min.) after Diamox	Appearance Times (sec.)
Controls		—	248±26 (10)
Diamox (mg./kg.)	50	15	328±24·5 (6)
	20	15	334±32 (5)
	50	60	348±21 (9)
	50	180	208±15 (9)
	50	360	187±30 (6)

A typical result of an animal given Diamox 1 hour previously is seen in Fig. 3. This shows the increased appearance time and also that the time taken for fluorescein to become evenly distributed in the anterior chamber exceeded that in a control experiment (Fig. 2). This latter observation was emphasized by the unequal distribution of fluorescein after 30 minutes in all animals given Diamox compared with the equal distribution of fluorescein observed in the majority of untreated animals (Langham and Lee, 1955a).

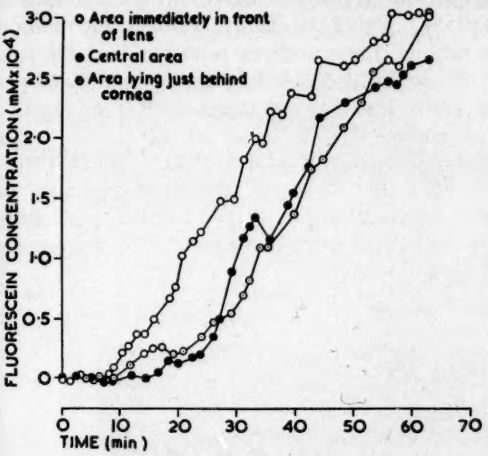

FIG. 3.—Concentration of fluorescein in aqueous humour in a rabbit given 50 mg. Diamox/kg. body wt 1 hr previously. 1·0 ml. of a 10 per cent. solution of sodium fluorescein was injected intravenously.

The dosage of Diamox given to the animals well exceeded that needed to reduce the intra-ocular pressure and it is considered that the observed effects on the appearance times reflect its maximum response. It was, however, considered of interest to test whether a low dosage would influence the appearance time since Gloster and Perkins (1955b) observed a lowering of the intra-ocular pressure in animals receiving 5 mg. Diamox/kg. body weight; this is also a similar dosage to that given to patients with glaucoma. In five rabbits given 5 mg. Diamox/kg. body weight 15 minutes previously, the mean appearance time was 300 ± 25 seconds, which significantly exceeds the value in untreated animals and is similar to that given greater amounts of the drug.

The effect of Diamox on rabbits in which the renal arteries had been tied was found more difficult to assess owing to an increased permeability of the iris vessels to fluorescein and protein in some animals. Control animals were given fluorescein 2 hours after tying the renal arteries and the mean appearance time was 218 ± 40 (5) seconds, which is similar to that found in normal animals. In similar experiments, Diamox (50 mg./kg.) was given 1 hour after the operation and fluorescein 1 hour later. The appearance time in these conditions was 280 ± 31 (7) seconds. Although this value

exceeds that in the control series, it cannot be considered statistically different in view of the large standard error.

In a final series of experiments, the fluorescein appearance time of rabbits given ammonium chloride by mouth 1 hour previously was determined. The mean value was 335±30 seconds and is significantly higher than the appearance time in untreated animals.

The observation that the rate of formation of the aqueous humour is decreased 1 hour after administration of Diamox agrees with the observations of Becker and Constant (1955). Using the electric tonographic method of Grant (1950) on conscious rabbits, these authors reported that the rate of formation of the aqueous humour was decreased by approximately 63 per cent. 45–60 minutes after intravenous injections of Diamox (100 mg./kg.).

Intra-Ocular Pressure Studies.—In a preliminary series of experiments, the observations of Gloster (1955) that Diamox given intravenously to rabbits reduces the intra-ocular pressure within a few minutes were fully confirmed. A typical result in which the intra-ocular and blood pressures were measured are shown in Fig. 4.

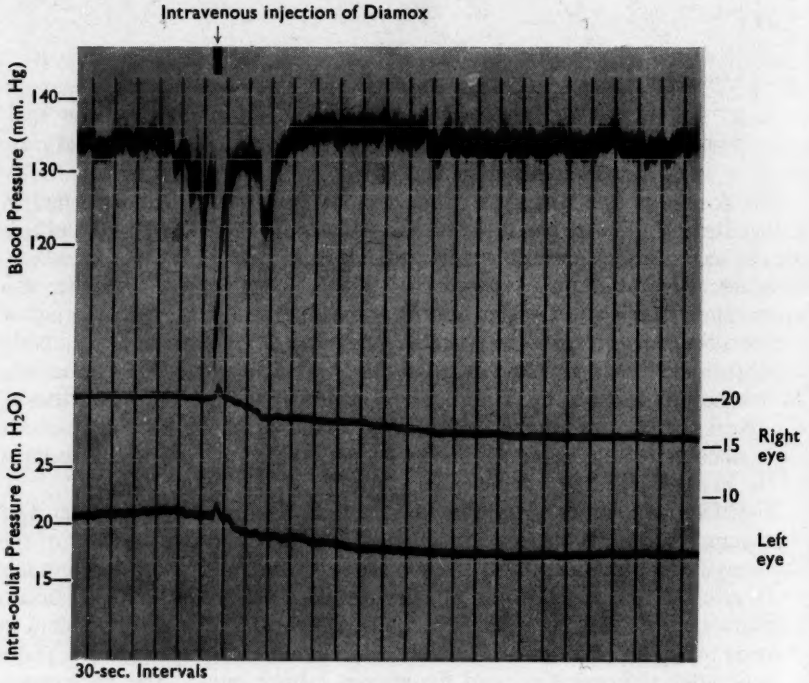

FIG. 4.—Effect of Diamox (25 mg./kg. body wt) given intravenously on intra-ocular pressure of a rabbit.

In similar experiments, Diamox was administered intravenously 2·5 hours after the administration of ammonium chloride, and again Diamox was found to reduce the intra-ocular pressure in all six animals studied. Fig. 5 records a typical result and shows that the fall in the intra-ocular pressure followed a pattern similar to that seen on animals not pre-treated with ammonium chloride. These observations confirm the findings of Becker (1955a), who measured the intra-ocular pressure of rabbits by a tonometric technique after the administration of ammonium chloride.

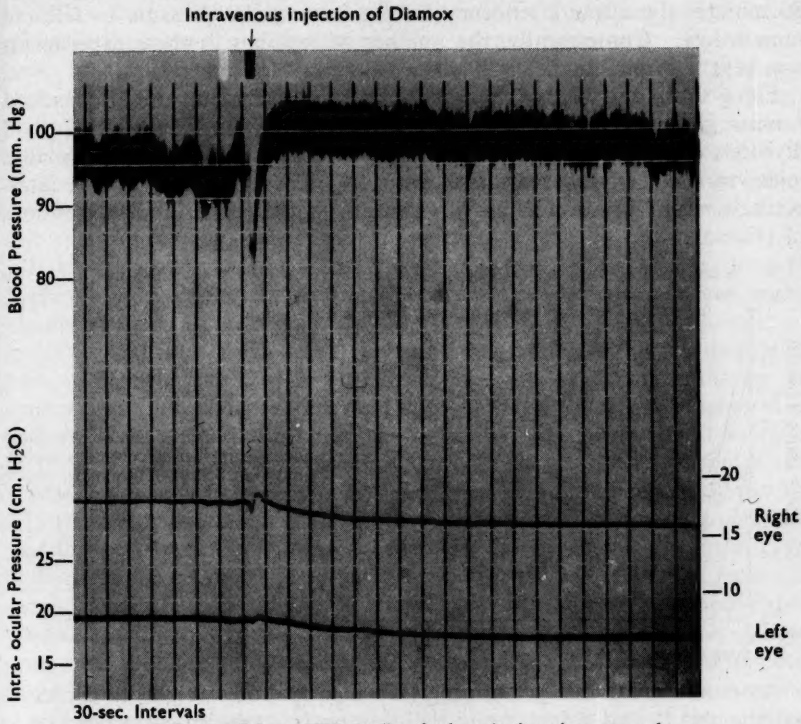

FIG. 5.—Effect of Diamox (25 mg./kg. body wt) given intravenously on intra-ocular pressure of a rabbit 2·5 hrs after oral administration of ammonium chloride (270 mg./kg. body wt).

The conventional Schiötz X tonometer was used to observe the influence of Diamox and ammonium chloride on the intra-ocular pressure over a period of several hours. The manometric technique could not be employed for, although it is more accurate than tonometry, it is unsuitable for recording slow changes in pressure in the eye. In a control series of experiments on anaesthetized rabbits, the intra-ocular pressure was found to fall slowly during a period of 5 hrs, and it was found increasingly difficult to obtain reproducible tonometric readings. Attempts were therefore made to record

tonometric readings on conscious rabbits. In these conditions it was found that the tonometric readings remained essentially unchanged in the two eyes during a period of 5 hours provided the application of local anaesthetic to the eye was kept to a minimum. To achieve an adequate anaesthesia of the corneal epithelium surface one drop of a 1 per cent. solution of pantocaine was instilled into the conjunctival sac and the excess was washed away with an isotonic saline solution 1 minute later. If, however, the local anaesthetic remained on the eye and readings were taken at intervals of 30 minutes there was a tendency for the intra-ocular pressure to fall and remain low. Consequently, the number of readings in these experiments was kept to a minimum.

Fig. 6 shows the relative changes in the intra-ocular pressure of conscious rabbits given Diamox. The pressure fell rapidly and averaged 2 to 3 divisions on the Schiötz scale within 30 to 60 minutes of the intravenous injection. Later, however, there was a significant recovery of the intra-ocular pressure towards its initial value in spite of further hourly injections of Diamox.

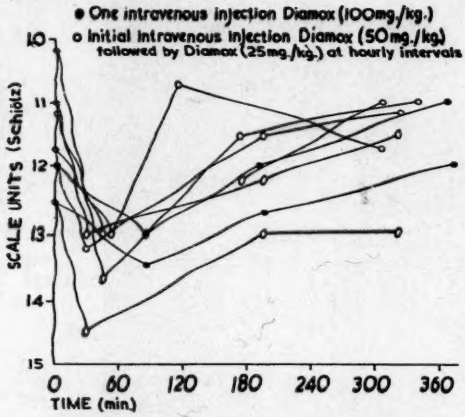

FIG. 6.—Effect of Diamox on intra-ocular pressure of conscious rabbits. Standard Schiötz X tonometer used to measure intra-ocular pressure.

Ammonium chloride given orally (270 mg./kg. body weight) to conscious rabbits also caused a decrease in the intra-ocular pressure corresponding to a change of 2 to 3 divisions on the Schiötz scale. In contrast to the effect of Diamox, the intra-ocular pressure remained low over the experimental period of 5 hrs. Attempts to confirm this hypotensive action of ammonium chloride by the manometric technique were not successful owing to its action on the blood pressure when injected intravenously.

Discussion

In assessing how Diamox influences the composition of the aqueous humour and intra-ocular dynamics several observations merit special mention. First, Diamox caused a reduction in *pH* and also in the concen-

trations of bicarbonate in the aqueous humour and the blood, but only a temporary reduction in *the ratio of the concentrations* of bicarbonate between the aqueous humour and the blood. Secondly, there was a rapid but only temporary decrease in the rate of flow of the aqueous humour. Thirdly, Diamox neither inhibited the secretion of ascorbic acid across the blood-aqueous barrier nor significantly affected the distribution of sodium between the two fluids. Lastly, there was a rapid and significant fall in the intra-ocular pressure followed by a partial or complete recovery. It would seem to follow that, in the continued presence of Diamox, there is little or no effect on the intra-ocular dynamics of the normal rabbit eye. At the same time, in confirmation of previous investigations, our experimental results show that Diamox can cause transient changes in the intra-ocular pressure and in the formation of the aqueous humour. For this reason, the animals employed in this study could not be considered resistant to the action of Diamox. These views are not, however, in agreement with the conclusions of Becker (1956) and Becker and Constant (1955); in this discussion the discrepancies may to some extent be resolved.

It now appears well established that in rabbits the concentrations of hydroxyl and bicarbonate ions in the aqueous humour exceed those in the plasma and that the two fluids are not in thermodynamic equilibrium in so far as the distribution of these two ions is concerned. In an ultrafiltrate of plasma, Greene and Power (1931) reported that the concentrations of hydroxyl and bicarbonate ions were approximately 4 per cent. higher than in the plasma water and in agreement with the theoretical van Slyke ratio of 1·04. In studies of the dialysis of horse serum against a solution of salt at 38° C., Hastings, Salvesen, Sendroy, and van Slyke (1927) reported ratios of 1·13 for the hydroxyl ion and 1·03 for the bicarbonate ion, and concluded that the bicarbonate ion approached more closely to the theoretical van Slyke ratio than chloride, sodium, and hydrogen ions. On this basis the excess of hydroxyl and bicarbonate ions in the aqueous humour above that consistent with passive ultrafiltration or dialysis of the plasma is 20–30 per cent. In absolute amounts the excess of bicarbonate in the aqueous humour was equal to 7mEq/kg.H_2O. This exceeds that observed for other ions and is well above that required to account for the hypertonicity of the aqueous humour which is generally agreed not to exceed 2 to 3 mMNaCl/kg.H_2O. Consequently, the excess bicarbonate must be partially or completely compensated by a reciprocal deficit of another anion.

The origin of the high concentrations of hydroxyl and bicarbonate ions in the aqueous humour remains unsolved. One explanation that either or both of these ions are secreted across the stromal epithelial barrier of the ciliary process was suggested by Friedenwald, Buschke, and Michel (1943) on the the evidence of the transfer of dyes across the ciliary processes. Friedenwald (1949) postulated that the primary product of the secretory mechanism was the accumulation of hydroxyl ions in the epithelium and

hydrogen ions in the stroma, and that an excess of bicarbonate ions would accumulate on the epithelial side of the barrier through the reaction of hydroxyl ions with carbonic acid. There is, however, an alternative explanation—that the excess of these ions is dependent on the composition and metabolic activity of the corneal, lens, and retinal tissues. The ions and water constituting the aqueous humour are freely diffusible with those in the cornea across the endothelium. Therefore, in considering the nature of the aqueous humour and its thermodynamic equilibration with the plasma, the membrane-effect acting across the corneal-aqueous barrier cannot be ignored. The extracellular volume of the corneal stroma has a high concentration of indiffusible anions and the concentration of sodium exceeds that in the aqueous humour by some 20 to 30 per cent. (Langham and Taylor, 1956). This membrane-effect would tend to increase the *p*H and the concentration of all anions in the aqueous humour of the anterior chamber, above that found in an ultrafiltrate of plasma. Thus, in the presence of this factor, the anatomical complexities of the posterior and anterior chambers the presence of a constant circulation of the aqueous humour, and the low maximal concentration gradient of $1{\cdot}2 \times 10^{-8}$ mEq of hydroxyl ion/kg. H_2O between the plasma and the aqueous humour, it might be considered surprising if the hydroxyl ion were in complete thermodynamic equilibrium with the plasma. It is, therefore, of particular interest that Davson and Luck (1956) have shown that the distribution of bicarbonate between the aqueous humour and the plasma varies in different species and that in some animals, including man, the concentration in the aqueous humour is less than that in the plasma.

The effect of Diamox on the *p*H and on the concentration of bicarbonate in the plasma is consistent with its recognized action in increasing the *p*H and excretion of bicarbonate in the urine. The fall in the concentration of bicarbonate in the plasma of 6 to 8 mEq/kg. H_2O from an initial mean value of 24 mEq/kg. H_2O agrees well with similar observations on dogs (Maren and others, 1954) and man (Galdston, 1955). This took place with doses of Diamox of 20 mg./kg body weight and was not increased at concentrations of 100 mg./kg body weight. The main decrease in the concentration of bicarbonate in the plasma took place within the first 2 hours, although it is interesting to note that at 15 minutes the concentration had not altered significantly (compare Tables I, II, and III). This would seem to indicate that the loss of bicarbonate through the kidney had not reduced the body bicarbonate sufficiently to affect the plasma concentration during this period. It is therefore of special interest that there was in the same 15-minute period an apparent fall in the *p*H of the blood and an increase in the arterial CO_2 tension. From general physiological principles it is unlikely that Diamox could cause a decrease in the *p*H and an increase in the CO_2 tension of the blood within 15 min. Therefore these analyses could reflect changes inthe *p*H of the blood at the time of withdrawal. This explanation is supported

by the calculation that the *p*H of the blood equals that in untreated animals if the CO_2 tension is assumed to equal that in the aqueous humour.

In the same initial period of 15 minutes the *p*H decreased and the CO_2 tension of the aqueous humour increased and, in addition, there was a marked decrease in the concentration of bicarbonate. This could well be due to the increased acidity, for the very low buffering power of the aqueous humour is determined almost completely by its bicarbonate content. On the other hand, it could be argued that the decrease is consistent with a hypothetical inhibitory effect of Diamox on a process of secretion of bicarbonate into the aqueous humour. This, however, appears unlikely, since the excess concentration of bicarbonate in the aqueous humour became re-established within 2 to 3 hours in spite of further injections of Diamox.

Thus, although Diamox caused a temporary disturbance of the normal distribution ratio for bicarbonate, the excess of both hydroxyl and bicarbonate ions in conditions of steady-state were proportionately the same as in normal untreated rabbits. It is therefore evident that Diamox, even though it may completely inhibit the carbonic anhydrase activity in the ciliary processes (Green, Capper, Bocher, and Leopold, 1954), does not alter the normal distribution ratios of these ions. Consequently, it is unlikely that carbonic anhydrase activity in the eye is essential to the accumulation of excess bicarbonate in the aqueous humour.

The report of Becker (1956) that the normal distribution of bicarbonate between the aqueous humour and the blood is altered by Diamox hinges on the fact that he did not detect a decrease in the concentration of bicarbonate in the plasma of rabbits given Diamox during a period of 6 hours. His results on the aqueous humour, which were determined in a similar manner to those described in this paper, are in accord with the present observations; and this strengthens our opinion that a similar agreement would have been obtained in the plasma analyses had the more detailed procedure used in this study been followed. It is to be remembered that a decrease in the concentration of bicarbonate in the plasma of animals given Diamox for several hours agrees with the observations of many workers in this field (*e.g.* Maren and others, 1954; Galdston, 1955), and indeed constitutes the accepted mode of action of Diamox in increasing the renal excretion of sodium bicarbonate (Berliner, 1951).

The analyses of the sodium content of the aqueous humour show that the marked loss of bicarbonate immediately after giving Diamox is not accompanied by an equivalent decrease in the concentration of the main cation, sodium. It would seem that there was no marked reduction in the rate of transfer of sodium into the eye either during this period or in conditions approaching steady-state. This conclusion agrees with the findings of Kinsey, Camacho, Cavanaugh, and Constant (1955), who reported that Diamox did not affect the turnover rate of sodium in the aqueous humour.

The constancy of the steady-state ratios for the sodium, hydroxyl, and bicarbonate ions lends support to the experimental evidence that the intraocular dynamics remain essentially unchanged after the administration of Diamox.

The transfer of ascorbic acid across the blood-aqueous barrier was studied in the belief that, if Diamox affected the secretory mechanism in the manner postulated by Friedenwald (1955a, b), the transfer of ascorbic acid would presumably also be modified. Friedenwald (1955a) suggested that carbonic anhydrase acts by facilitating the neutralization of hydroxyl ions on the epithelial side and hydrogen ions on the stromal side of the ciliary process. In the former area the excess of hydroxyl ions would react with carbonic acid to form bicarbonate, and in the latter area hydrogen ions would react with bicarbonate to form carbonic acid. Diamox, it was postulated, would slow down these reactions of neutralization and lead to a change in *p*H which could inhibit the enzymes necessary for the secretory process. Now ascorbic acid is the only compound which is universally agreed to be secreted across the ciliary processes, and to it has been ascribed a cardinal role in the secretory mechanism (Friedenwald, 1949). It is therefore of some significance that the transfer of this compound across the blood-aqueous barrier underwent no significant change in the presence of Diamox. It is true that the small increase in the concentration of ascorbic acid in the aqueous humour of animals given Diamox 3 hours previously is consistent with an increase in the rate of secretion; but, on the other hand, it may be more simply ascribed to the transient decrease in the rate of flow of the aqueous humour during the first 1 or 2 hours.

The results with ascorbic acid are in general agreement with those reported by Becker (1956) in nephrectomized rabbits. In this paper he analysed the concentration of ascorbic acid in the posterior and anterior chambers and in the plasma, and used these values to derive information about the rate of flow of the aqueous humour. This approach was based on the equations of Kinsey and Palm (1955) in which the rate of accumulation of a substance in the anterior chamber was expressed in the form

$$\frac{dC_{aq}}{dt} = K_f(C_\pi - C_a) + K_d(C_p - C_a),$$

Where C_π, C_a, and C_p represent, respectively, the momentary concentrations of the test substance in the fluid passing through the pupil ($_\pi$), in the anterior chamber (a), and in the plasma (p). K_f represents the rate of flow per minute as a fraction of the volume of the anterior chamber; K_d represents the corresponding rate of diffusional exchange between the anterior chamber and the plasma. In conditions of steady-state,

$$\frac{dC_a}{d_t} = \text{zero}, \quad \text{and} \quad \frac{K_f}{K_d} = \frac{C_a - C_p}{C_\pi - C_a}.$$

Using both equations, Becker found that, in animals given Diamox, the rate flow of the aqueous humour was depressed 60 per cent. in conditions of steady-state.

This conclusion is in apparent disagreement with the observations recorded in the present study that the rate of flow is unchanged in conditions of steady-state. It should, however, be pointed out that the experiments of Becker were carried out on nephrectomized rabbits; the conclusion that Diamox inhibits the rate of flow of the aqueous humour by 60 per cent. in normal animals rests on the assumption that rabbits in which the renal arteries have been ligated 18 to 24 hours previously are normal. This, however, is not so, for the present experiments show that changes occurred in the composition of the aqueous humour, in the permeability of the blood-aqueous barrier, and in the intra-ocular pressure of rabbits after ligation of the renal arteries. Furthermore, Diamox caused an irreversible lowering of the intra-ocular pressure in these animals.

There is general agreement that fluorescein can be used as a test substance to measure relative changes in the rate of flow or in the formation of the aqueous humour (Linnér and Friedenwald, 1954; Becker, 1955b; Langham and Wood, 1956). The experimental results in this paper showed that the time taken for fluorescein to pass from the bloodstream and into the anterior chamber *via* the posterior chamber increased by approximately 50 per cent. 14 minutes after the administration of Diamox, and that this increase in the time of flow remained high for at least 1 to 2 hours. Subsequently, however, there was a return of the flow-time to normal in spite of the administration of further amounts of Diamox. An increase in the flow-time of 100 per cent. would be equivalent to a decrease in the rate of flow of at least 50 per cent., and the observed increase of 50 per cent. in appearance time would be equivalent to a decrease in the rate of flow of approximately 20–25 per cent. The changes in the rate of flow of the aqueous humour after the administration of Diamox is of interest, in that the initial decrease in the rate of flow occurred at the same time as the loss of bicarbonate in the aqueous humour. Furthermore, the recovery of flow to normal was concurrent with the return of the bicarbonate distribution ratio to normal. Presumably the action of Diamox in decreasing the rate of flow of the aqueous humour is associated with a disturbance of the normal osmotic balance between the aqueous humour and the blood, and these results are consistent with the view that the loss of bicarbonate in the aqueous humour was responsible for the temporarily decreased rate of formation.

The variation observed in this study in the level of the intra-ocular pressure in normal rabbits given Diamox is in agreement with the results of previous investigations. The change was rapid and reached a maximum within 30 to 60 minutes. Becker (1955a) and Becker and Constant (1955) reported that the normal intra-ocular pressure in rabbits fell rapidly and reached a minimal value within 20 to 40 minutes and that in some rabbits the intra-

ocular pressure returned to normal within 2 hours. By the tonographic technique of Grant (1950), they found evidence that during this time there was in increase in the resistance to outflow which tended to minimize the full action of the Diamox. This increase in the resistance to outflow cannot however explain the return of the intra-ocular dynamics to normal in conditions of steady-state, for if the rate of formation is unchanged, an increase in the resistance to outflow would cause the intra-ocular pressure to rise above its normal value. It is therefore likely that any change in the resistance to outflow takes place only when the intra-ocular pressure and the rate of formation of the aqueous are depressed during the period immediately following the administration of Diamox.

The conclusion emerging from these studies—that the intra-ocular dynamics of the normal eye are almost, if not completely, undisturbed by Diamox in conditions of steady-state—is not inconsistent with the well-proven fact that, in similar conditions, the intra-ocular pressure is decreased and controlled in the glaucomatous eye. It is reasonable to assume that the immediate action of Diamox on the body is basically the same, and that the essential difference in the final equilibrium is determined by the different properties of the hypertensive eye. Theoretically, there are several factors which could make the eye with a high intra-ocular tension react more dramatically than the normal eye. First, there is the obvious reason that a given change in flow will lead to a greater absolute fall in the intra-ocular pressure if the initial pressure is high. Secondly, there is the possibility that homeostatic mechanisms are operating in the eye with a normal intra-ocular pressure and not in the eye with a pressure above a certain critical level. In this case, Diamox may reduce the pressure sufficiently to the level at which the homeostatic mechanism acts. Thirdly, it is possible that the relative contributions of filtration and osmotic pressures in the maintenance of the flow of the aqueous humour are not the same in the two cases. In this event Diamox could affect the rate of formation of the aqueous humour in the normal and abnormal eye disproportionately.

There are few reports of the action of Diamox on normal human eyes. Grant and Trotter (1954) observed that Diamox in doses used clinically did not decrease the intra-ocular pressure of normal eyes more than 1 or 2 mm. Hg, and they also made the interesting observation that in glaucomatous eyes which were at comparable initial pressures due to the action of miotic drugs, the change in intra-ocular pressure after Diamox was only 3 mm. in four out of five eyes. These results referred to conditions of steady-state. Similar observations on normal and glaucomatous human eyes have been reported by Campbell, Tonks, and Jones (1956). The lack of change in the intra-ocular pressure of these subjects contrasts with the observations of Becker (1955c) on fifteen normal eyes of ten subjects given a high dosage of Diamox by mouth. Approximately 3 hours after giving 500 mg. Diamox by mouth the intra-ocular pressure was found to be decreased from a mean value of

17·6 to 14·6 mm. Hg, and the rate of flow to be decreased by a mean value of 55 per cent. as measured by the tonographic technique.

The assessment of how and to what extent Diamox acts on the eye is handicapped by our lack of knowledge of the contribution that secretion and the hypertonicity of the aqueous humour play in the movement of water through the anterior chamber. It is widely accepted that the osmotic pressure of the aqueous humour is 1 to 2 per cent. higher than that of the blood (Benham, Duke-Elder, and Hodgson, 1938; Roepke and Hetherington, 1940; Davson, Duke-Elder, and Maurice, 1949; Bárány, 1947; Kinsey, 1951). This osmotic excess is equivalent to a hydrostatic pressure of 100–200 mm. Hg. and would mean that the hydrostatic pressures acting across the blood-aqueous barrier contributes in a minor way to the movement of fluid through the eye. On the other hand, the estimate of the osmotic pressure of the aqueous humour may be high, since these determinations were made at temperatures below 37·5° C. Now the *p*H-temperature coefficient is not the same for blood and for the aqueous humour and, as a consequence, a given decrease in temperature will disturb the two fluids disproportionately. In this connexion, the studies of Ingraham, Lombard, and Visscher (1933) have shown that the distribution ratio for sodium and potassium ions between plasma and its ultrafiltrate decreases with an increased *p*H; the value for sodium fell from 0·928 to 0·913 and for potassium from 0·901 to 0·816 when the *p*H increased from 7·0 to 7·70. Consequently, in determinations of the relative osmotic activity of these two fluids at temperatures below that of the body, an error is introduced which causes an increase in the osmotic pressure of the aqueous humour in relation to that of the blood.

In bringing together these findings on normal and glaucomatous eyes, the evidence is consistent with the view that Diamox modifies the osmotic balance between the blood and the aqueous humour independently of its renal action. The mechanism remains unsolved, but would appear to be related to an increased acidity in the eye. In animals, however, this increased acidity relative to that of the blood was only temporary. This could have been due to a disproportionate inhibition of carbonic anhydrase activity in different regions of the eye through a slow penetration of Diamox, or it could have reflected the ability of the eye to reassert the normal concentration ratios of hydroxyl and bicarbonate ions between the aqueous humour and the blood. Apart from the changes in the concentration of bicarbonate in the aqueous humour immediately after giving Diamox, no evidence has been found to support the view that Diamox inhibits the secretion of bicarbonate into the eye. The possibility that Diamox directly or indirectly inhibits the active transport of some other ion such as sodium or chloride cannot be answered from this study.

Summary

(1) The *p*H and the concentration of total carbon dioxide in the aqueous humour and in the arterial blood of normal rabbits have been determined. The *p*H temperature coefficient was determined separately and found to be 0·0147±0·0002 and 0·0064±0·00025 *p*H units per ° C. for the blood and the aqueous humour respectively. The *p*H and the concentration of bicarbonate in the aqueous humour exceeded those in the blood and the calculated values of the pCO_2 tensions in the two fluids were found to be equal.

(2) The composition of the aqueous humour and the blood has been analysed at varying times after an injection of Diamox. A rapid fall in the *p*H and a concurrent increase in the CO_2 tension in the two fluids was observed. At the same time there was a decrease in the concentration of bicarbonate in the aqueous humour and a temporary decrease in the normal excess of bicarbonate. In conditions of steady-state, however, the distribution ratio (C_{aq}/C_{pl}) was equal to that in control animals.

(3) Ammonium chloride, given in amounts that produced a marked acidosis, resulted in a prolonged decrease in the excess of bicarbonate in the aqueous humour. Ligation of the renal arteries induced a state of progressive acidosis, but left the normal distribution ratio for bicarbonate unchanged. The administration of Diamox to these animals resulted in a further increase in the CO_2 tension and a decrease in the *p*H of the aqueous humour.

(4) Analysis of the concentrations of sodium in the aqueous humour and the blood after administration of Diamox showed that the initial fall of approximately 6 mEq HCO_3/kg. H_2O in the aqueous humour was not accompanied by any significant change in the normal distribution ratio of the sodium ion.

(5) The effect of Diamox and ammonium chloride on the secretion of ascorbic acid across the blood-aqueous barrier has been studied. Both compounds led to an increase in the concentration of ascorbic acid in the aqueous humour compatible with a decrease in the rate of flow of the aqueous humour. It may be concluded that neither compound inhibited secretion of ascorbic acid.

(6) The circulation of the aqueous humour and its rate of flow in rabbits given Diamox or ammonium chloride have been studied using fluorescein as a test substance. The time taken for fluorescein to pass from the bloodstream through the posterior chamber to the pupillary area immediately in front of the lens was 238±23 (11) seconds; 1 hour after the administration of Diamox this increased to 348±21 (9) seconds. In animals maintained on Diamox for 6 hours, the appearance-times were normal. Ammonium chloride also led to an increase in the fluorescein appearance-time which

was maintained over several hours. It is concluded that Diamox temporarily reduced the rate of flow of the aqueous humour.

(7) Intra-ocular pressure observations, using both the manometric and the tonometric methods of measurements, are reported. In confirmation of other investigations, the intra-ocular pressure was found to fall rapidly after giving Diamox and then to increase toward normal. Ammonium chloride in doses that gave frank acidosis caused a more prolonged lowering of the intra-ocular pressure.

(8) It is concluded from these studies that the continued presence of Diamox gives rise to little or no effect on the intra-ocular dynamics of the normal rabbit eye. It is also suggested that the temporary reduction in the intra-ocular pressure, the rate of formation of the aqueous humour, and the excess of bicarbonate in the aqueous humour immediately following the administration of Diamox are associated with the action of this substance in inducing a rapid fall in the *p*H of the aqueous humour.

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DISTENSIBILITY OF THE EYE*

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IN clinical ophthalmology the intra-ocular pressure can rarely be determined directly, and reliance must be placed on measurements of the tension of the coats of the eye. The instruments used for the estimation of ocular tension (tonometers) measure the depth of indentation of the globe produced by a known weight. The depth of indentation depends on several factors, the most important of which are the intra-ocular pressure and the distensibility of the eye. Variations in distensibility can have a large influence on the calibration scales of tonometers and therefore some knowledge of the relationship between changes in volume in the eye and the resultant changes in intra-ocular pressure is necessary.

Schultén (1884), Koster (1895, 1901), Greeves (1913), Ridley (1930), and Clark (1932) investigated such volume-pressure relationships in the eyes of various species and, in spite of some differences in detail, the common feature of all the results of these investigators was that the change in pressure induced by a given change in volume in an eye was not constant but depended on the initial intra-ocular pressure. Smaller changes in pressure resulted from the same change in volume at lower intra-ocular pressures than at higher intra-ocular pressures. Friedenwald (1937) attempted to derive an expression relating the pressure and volume changes which could be applied over a wide range of intra-ocular pressures. In an analysis of the results of Koster, Ridley, and Clark, he claimed that above pressures of 5 mm. Hg a linear relationship existed between the volume increment and the logarithm of the pressure. Accepting this relationship, he formulated an equation:

$$\text{Log}\,\frac{P}{P_o} = K.\Delta V,$$

where P_o = initial intra-ocular pressure above 5 mm. Hg,

P = final intra-ocular pressure,

ΔV = volume change,

and K is a constant.

The constant K in this formula Friedenwald called the "coefficient of rigidity".

In the course of an investigation into the changes in intra-ocular pressure

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caused by changes in blood volume within the eye, we obtained results which suggested that the value of K was not constant in the rabbit eye, and the following investigation into the relationship between pressure and volume changes was therefore undertaken.

Method

The experiments consisted essentially in the measurement of intra-ocular pressure before and after the introduction of a known volume of fluid (a 0·9 per cent. solution of sodium chloride) into the eye. As fluid is able to leak from the eye through the normal drainage channels for aqueous humour, it is essential that these measurements be made as rapidly as possible.

Adult rabbits weighing 1·7 to 4·7 kg. were anaesthetized by the intravenous injection of a 25 per cent. solution of urethane in doses of 1·75 g./kg.

Two methods have been employed: the 'manometric' and the 'volumetric'.

(*I*) *Manometric Method.*—Fig. 1 is a diagram of the apparatus. Two needles *N1* and *N2* (15 gauge) were introduced into the anterior chamber of the eye of a rabbit after the application of one drop of 2 per cent. pantocaine into the conjunctival sac. The needle *N1* was connected by rigid polythene tubing (1 mm. internal diameter) to a pressure-measuring device, which consisted of a rubber membrane manometer *M* recording optically on a moving photographic film. A saline reservoir *R* was connected to this system by means of a tap *T*. The needle *N2* was connected directly to a micrometer syringe *S* (Agla) with an adjustable stop which allowed rapid rotation of the micrometer head by a predetermined amount. By this means a known volume of fluid (between 7 and 8μl.) could be introduced rapidly into the eye and associated manometric system.

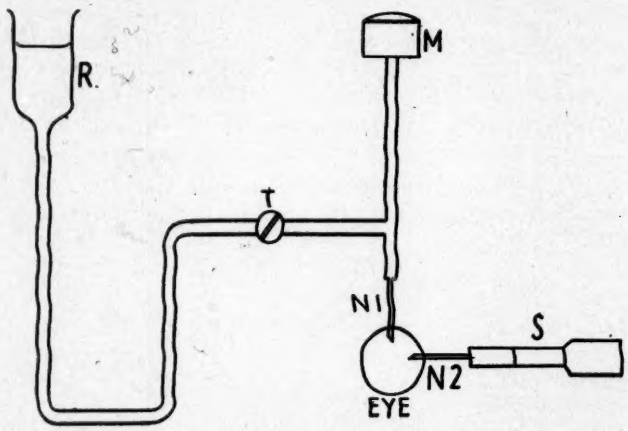

FIG. 1.—Diagram of apparatus for determination of ocular distensibility by manometric method.

M	Rubber membrane manometer	*R*	Saline reservoir
*N*1, *N*2	No. 15 gauge needles	*S*	Micrometer syringe
		T	Tap

The procedure was as follows: the tap *T* was turned to connect the reservoir *R* with the whole system, and the height of the reservoir was adjusted to the initial pressure—usually 5 to 10 cm. saline. The tap was turned to exclude the reservoir and saline was injected from the micrometer syringe. After 5 seconds the micrometer head was turned back to the starting position, and after a further interval of 5 seconds the process was repeated. From three to five injections and withdrawals were made at each pressure level, and the experiment was repeated at ascending pressure steps of 5 or 10 cm. saline up to 50 cm. saline and in similar descending steps to the initial pressure.

The animal was then killed with an overdose of nembutal intravenously and the entire experiment was repeated in the dead animal.

Finally, a calibration of the manometer was recorded on the photographic tracing by connecting the reservoir *R* directly to the manometer and raising *R* in steps of 5 cm. over the range of pressures involved. It was also necessary to determine how much fluid was taken up by the distension of the manometer membrane so that this could be subtracted from the volume injected from the micrometer syringe in order to calculate the volume of fluid which distended the eye. The manometer was therefore connected directly to the micrometer syringe and successive injections of 5μl. saline were made until the movement of the manometer membrane had covered the same range as obtained in the experiments.

Fig. 2 gives an example of a record obtained by this method, showing the changes in intra-ocular pressure following the injection and withdrawal of saline at seven different initial pressures. Using the calibrations of pressure and volume for the manometer, and knowing the total volume injected from the micrometer syringe, the rise of pressure in the eye and the corresponding increase in intra-ocular volume could be calculated.

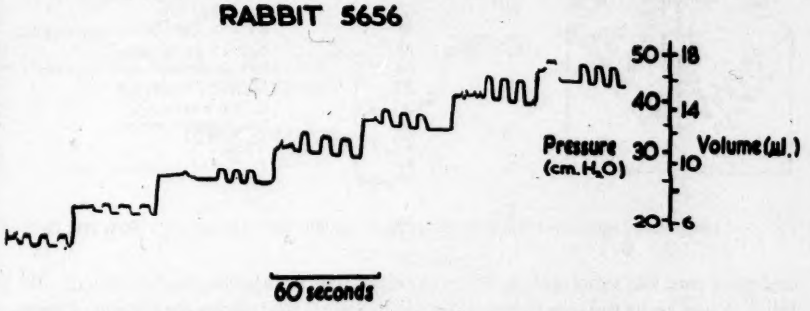

FIG. 2.—Record obtained by manometric method showing variations of intra-ocular pressure after repeated injection and withdrawal of fluid at seven pressure levels. (Pressure tracing line accentuated by re-touching with indian ink.)

(2) *Volumetric Method.*—A diagram of the apparatus is shown in Fig. 3 (overleaf). The volume of fluid entering the eye was measured by means of an apparatus which gave a continuous record of the flow of saline through a glass tube. The chief advantage of this method is that the rate of leakage of fluid from the eye is recorded and, if necessary, a correction can be applied for this. The flowmeter consisted of a glass tube *G*, 40 cm. in length, with an internal diameter of 1 mm., having side-pieces *P1* and *P2* at each end. A fine platinum wire *W*, having a resistance of 0·5 ohm/cm. extended through the lumen of the tube and was sealed in at either end and connected as part of a Wheatstone bridge circuit. One sidepiece *P1* of the glass tube was connected to reservoir *R1* which contained mercury, and the other sidepiece *P2* was connected to a second reservoir *R2* containing saline. The tube *G* was then filled, partly with saline and partly with mercury,

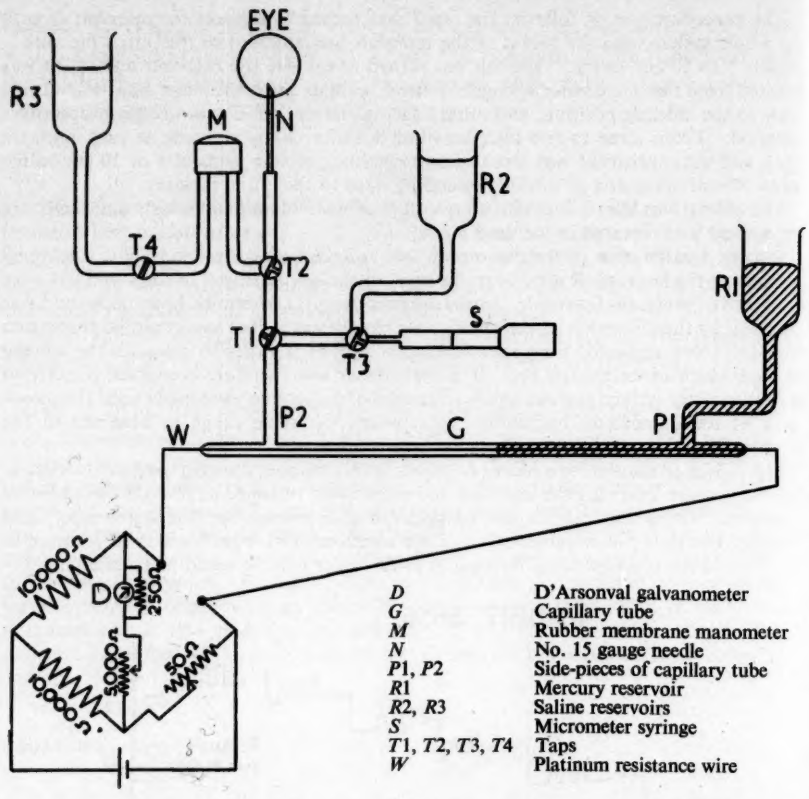

FIG. 3.—Diagram of apparatus for determination of ocular distensibility by volumetric method.

and great care was taken during filling to ensure that air bubbles were excluded. When saline flowed along the tube (from right to left in Fig. 3) the advancing column of mercury decreased the effective resistance of the wire *W*, and this was recorded on a photographic film from the mirror of a D'Arsonval galvanometer *D*.

The sidepiece *P2* was connected *via* the three-way taps *T1* and *T2* to a needle *N*, which was inserted in the anterior chamber of the eye, and also to the three-way tap *T3*, which was in turn connected to the saline reservoir *R2* and a micrometer syringe *S*. By means of a rubber membrane manometer *M*, connected to the anterior chamber *via* the tap *T2*, the intra-ocular pressure was recorded on the same photographic film as the galvanometer deflections. A third reservoir *R3*, filled with saline, could be connected to the system through the tap *T4*.

All connexions were made with polythene tubing and were as short as possible. The rigidity of the system was such that, when the needle *N* was sealed and *R2*, *R3*, *M*, and *S* were excluded from the system (as under the experimental conditions), the pressure could be increased from 0 to 70 cm. saline by raising the reservoir *R1* without any detectable displacement of the boundary between saline and mercury in the tube *G*.

The procedure was as follows:

The needle *N* was inserted into the anterior chamber of the eye of an anaesthetized rabbit with the tap *T2* open to the manometer and the reservoir *R3* set at 25 cm. above the eye and also connected to the manometer *via* the tap *T4*. By using the micrometer syringe *S*, the mercury saline junction was adjusted to a suitable position in the tube, and *T1* was turned to exclude the syringe and *R2* from the system; *R3* was now adjusted to the required pressure and taps *T2* and *T4* were turned to join *R3*, the manometer, and the eye to the tube *G*. *R1* was then raised to give a slow movement of the mercury along the tube. *T2* and *T4* were then turned to exclude the manometer *M* and *R3* from the remainder of the system. After allowing a few seconds for the galvanometer to record the position of the mercury saline junction, the reservoir *R1* was raised rapidly. The resulting influx of saline into the eye caused a movement of mercury along the tube which was recorded on the photographic film from the change in deflection of the galvanometer. *T2* was then opened to the manometer to record the new pressure in the eye. Fig. 4 shows an example of part of a record obtained by this method.

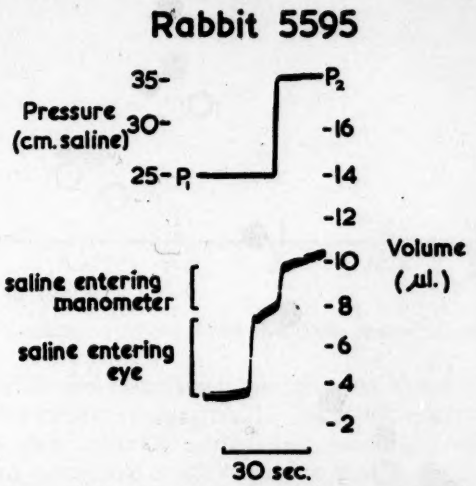

FIG. 4.—Record obtained by volumetric method showing volume of fluid entering eye for rise of intra-ocular pressure of *P*1, to *P*2 cm. saline. (Pressure tracing line accentuated by retouching with indian ink.)

The procedure was repeated at various levels of pressure and again after killing the animal. At the end of each experiment the tracing was calibrated for pressure and volume, using *R3* for the manometer, and the syringe *S* to obtain changes in volume in steps of 5μl. in the flowmeter tube.

Results

Five eyes were studied using the manometric method and five using the volumetric method, but the results from both methods were essentially the same and will be discussed together.

For all ten eyes the increase in intra-ocular pressure for unit volume of fluid injected into the eye became greater as the pressure at which the

measurements were made increased. The results of one experiment are shown in Fig. 5.

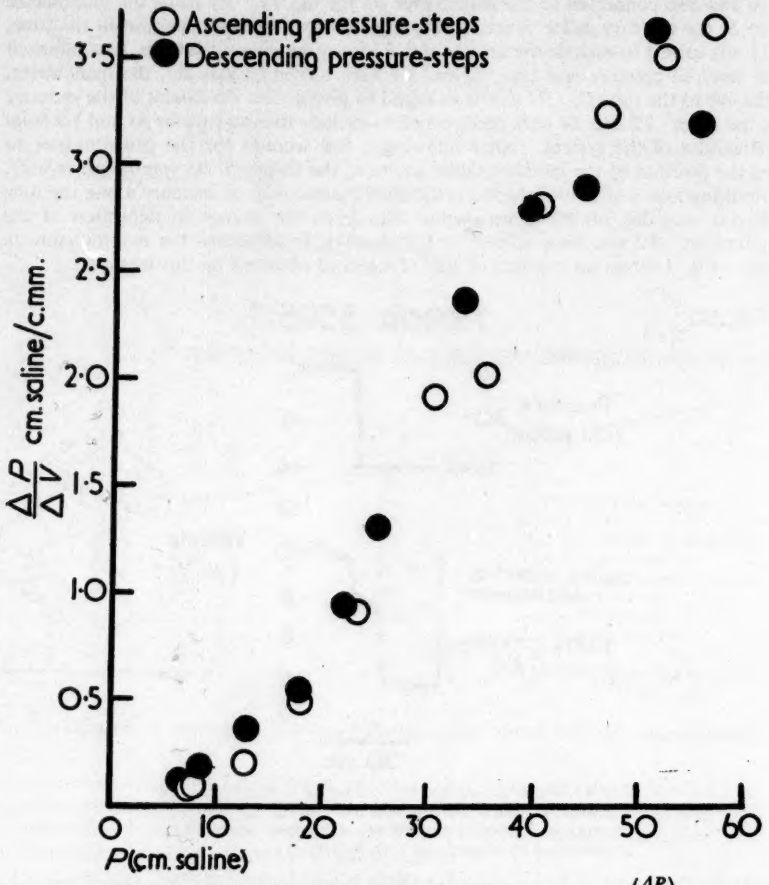

FIG. 5.—Relationship between pressure increment per unit volume injected $\left(\frac{\Delta P}{\Delta V}\right)$ and intraocular pressure (P=mean of initial and final pressures).

The coefficient of rigidity K was calculated at each pressure level from the formula:

$$K=\frac{\log_{10} P-\log_{10} P_o}{\Delta V},$$

where P_o was the initial pressure (cm. saline) and P was the pressure after injection of a volume of fluid, ΔV(μl.).

Fig. 6 (opposite) shows the results of one experiment, and it is seen that

K increased as the pressure at which it was determined increased.

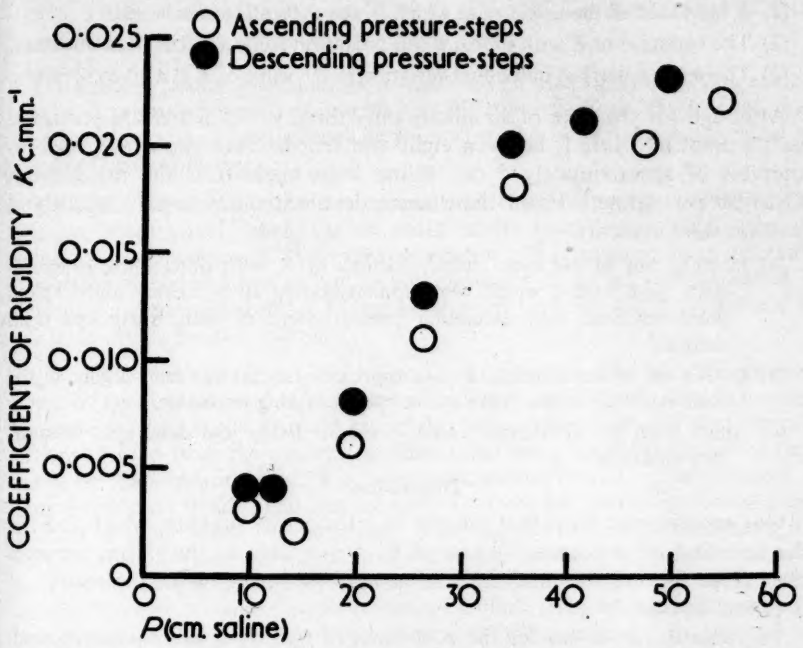

FIG. 6.—Variation of coefficient of rigidity *K* with intra-ocular pressure (*P*=mean of initial and final pressures).

The results for all ten experiments are given in Table I, which shows values for *K* in three pressure ranges, *viz.* 15–25, 30–40, and 45–55 cm. saline; if more than one observation fell within each range, the value of *K* at a pressure nearest to the middle of the range was chosen.

TABLE I

VARIATION OF COEFFICIENT OF RIGIDITY *K* WITH INTRA-OCULAR PRESSURE IN THE LIVING RABBIT EYE

Rabbit No.	Coefficient of Rigidity *K* (c. mm.$^{-1}$)		
	Pressure Range 15–25 cm. Saline	Pressure Range 30–40 cm. Saline	Pressure Range 45–55 cm. Saline
1	0·005	0·010	0·013
2	0·003	0·008	0·011
3	0·006	0·018	0·020
4	0·013	0·019	0·022
5	0·006	0·019	0·023
6	0·011	0·031	0·028
7	0·007	0·010	0·019
8	0·005	0·008	0·015
9	0·003	0·007	0·007
10	0·011	0·023	0·025

The main conclusions to be drawn from these results are as follows:

(1) K increased as the pressure at which it was determined increased.

(2) The variation of K with pressure was greater in some animals than in others.

(3) There was a marked individual variation in the value of K at a given pressure.

Although for the sake of simplicity only three values for K are given for each animal in Table I, between eight and ten determinations at pressure-intervals of approximately 5 cm. saline were made over the range from 10 to 50 cm. saline. From these more detailed results certain additional features were evident:

(*a*) In eight out of ten eyes, determinations of K with descending pressure-steps gave values which were approximately 20 per cent. higher than those obtained with ascending pressure-steps in both living and dead animals.

(*b*) In five out of ten animals, K was approximately 20 per cent. higher in the dead eye than in the living eye at corresponding pressures.

(*c*) Apart from the differences noted in (*b*) the living and dead eyes behaved very similarly.

Discussion

Our experiments show that the rise in intra-ocular pressure which follows the injection of the same volume of fluid increases as the initial pressure rises (Fig. 5), and this finding is in general agreement with the results of previous workers.

In Table II our values for the coefficient of rigidity K are compared with those calculated from the data of other workers. Whilst it is evident that our values for K for the rabbit eye are in general agreement with the results of previous investigations, our finding that K increases as the intra-ocular pressure rises (Fig. 6 and Table I) does not agree with Friedenwald's contention that this coefficient is constant for any individual eye above a pressure of 5 mm. Hg.

TABLE II
COEFFICIENT OF RIGIDITY K FOR THE EYE OF THE RABBIT

Author and Date	K (c.mm.$^{-1}$)	Pressure Range (cm. saline)	No. of Animals	State of Eyes
Present study	0·003–0·028 0·003–0·036	15–55 15–55	10 10	Living Dead *in situ*
Schultén (1884)	0·014–0·028	15–55	5	Enucleated
Koster (1895)	0·005–0·027	15–55	1	Enucleated
Ridley (1930)	0·004–0·010	3–100	6	Dead *in situ*
Grant and Trotter (1955) ..	0·019–0·022 0·012–0·024	Not stated Not stated	1 2	Living Dead *in situ*

Friedenwald (1937) based his calculations mainly on the work of Koster (1895, 1901), Ridley (1930), and Clark (1932), and a critical review of their methods and results reveals the following facts:

(1) Koster's results give values for K which have a wide variation. Consecutive results at pressure intervals of 5 or 10 mm. Hg differ by 50 to 300 per cent., and it is therefore impossible to conclude from them that K remains independent of the intra-ocular pressure.

(2) Ridley briefly described two methods for determining the distensibility of the eye, but it would appear that in neither method was it possible to measure small volume changes accurately, the inaccuracies thus introduced being especially important at higher levels of intra-ocular pressure. Even so, calculations of K from the graphs published by Ridley show some tendency for K to increase as the intra-ocular pressure increases.

(3) Clark used the eyes of the dog, cat, and macaque, and calculations from some of her data show that K tended to increase slightly with the intra-ocular pressure over the range 10 to 50 cm. saline. She did not consider that the rate of loss of fluid from the eye was significant and made no reference to the time taken for each measurement of pressure and volume change. The values of K (dog 0·0015, cat 0·0017, and macaque 0·0041) from her results are low in comparison with our values for the rabbit. This suggests that she overestimated the volume of fluid which distended the eye, because her experimental method may have permitted leakage of a significant amount of fluid through the angle of the anterior chamber. Since the rate of leakage of fluid from the eye is proportional to the intra-ocular pressure, overestimation of the volume of distending fluid would be accentuated at higher pressures. Therefore, the values for K, calculated from Clark's data, are probably too low at higher pressures, and any increase in K with the increase of intra-ocular pressure is partially obscured.

Results for the rabbit eye were also obtained by Schultén (1884), but Friedenwald made comparatively little use of these beyond mentioning that graphical analysis demonstrated an approximately linear relationship between the logarithm of the intra-ocular pressure and the volume increment. However, when K is calculated from Schultén's data, it is found to increase progressively from 0·014 at 17 cm. saline to 0·028 at 48 cm. saline.

It is clear therefore that Friedenwald's assumption that the coefficient of rigidity K is independent of the intra-ocular pressure was based on experimental work, some of which is open to criticism, and that his conclusions therefrom are open to question.

Our results show that the relationship between pressure and volume changes in the eye is complex. This could be due to the fact that the coats of the eye consist of two main layers: the highly vascular choroid, and the almost avascular sclera and cornea. It is conceivable that the choroid could exert a "cushioning" effect in the eye by virtue of its vascularity and connexions with the venous system outside the eye. In the dead eye such an

effect is excluded, and the similarity of the variation of K with intra-ocular pressure in the dead eye and in the living eye makes it very unlikely that the variation found by us is due to the influence of the choroid. On the other hand, the lower values for K in the living eye as compared with the dead eye may be attributable to a "cushioning" effect of the choroid. The sclera does not have a uniform thickness or structure throughout its extent, and Ischreyt (1899) demonstrated that the extensibilities of strips of sclera taken from different meridians of the globe were not the same. Furthermore, it has been observed previously by Ridley (1930) that the maintenance of the high intra-ocular pressure alters the distensibility of the eye; this is the probable explanation of our higher values for K in both living and dead eyes when determined with descending pressure-steps as compared with ascending pressure-steps. In view of the complex physical properties of the sclera, it is not surprising that the relationship between intra-ocular pressure and volume does not conform to a simple law.

This is a question of considerable importance from both the experimental and the clinical point of view, and it is obvious that it merits further exploration.

Summary

(1) Two methods for investigating the relationship between changes in pressure and volume in the rabbit eye are described.

(2) The increase in intra-ocular pressure per unit volume of fluid injected into the eye becomes greater as the intra-ocular pressure increases.

(3) Friedenwald's coefficient of ocular rigidity K was calculated from the data. Contrary to Friedenwald's assertion, this coefficient was not constant, but increased with the intra-ocular pressure in both living and dead eyes.

(4) Measurements of K in dead eyes sometimes gave higher values than in living eyes at corresponding intra-ocular pressures. In the majority of eyes, both living and dead, K was found to be slightly higher after the eye had been subjected to a raised intra-ocular pressure.

(5) It is suggested that the complexity of the relationship between changes in pressure and volume in the eye depends largely on the physical properties of the sclera.

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EXTENSIBILITY OF STRIPS OF SCLERA AND CORNEA*

BY

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THE relationship between changes of volume and changes of pressure in the eye is of considerable importance in the theory and practical application of tonometry and tonography. On the basis of the experimental evidence of earlier workers, Friedenwald (1937) claimed that this relationship could be expressed by the equation:

$$\text{Log}\,\frac{P}{P_0}=K\Delta V,$$

Where P_0=Initial pressure

P=Pressure after a change in intra-ocular volume ΔV

K=a coefficient of ocular rigidity.

Friedenwald stated that the value of K remained constant for individual eyes above a pressure of 5 mm. Hg.

In a recent study (Perkins and Gloster, 1957), however, it has been found that for the rabbit eye this fundamental assumption is invalid, for K increased as the pressure at which it was determined increased (Fig. 1, overleaf). Indeed, we were unable to formulate a simple expression for the relationship between changes in volume and changes in pressure in the eye of this animal, and suggested that a study of the extensibility of the sclera and cornea might help to explain our observations.

Little work appears in the literature on this problem. Schelske (1864) gave figures for a coefficient of elasticity for the cornea and sclera of human and rabbit eyes. In human eyes he found that there was a small difference (approximately 7 per cent.) between the coefficient for meridional and equatorial strips of sclera, but that the cornea was more than twice as extensible as the sclera. In the rabbit the cornea was found to be only some 20 per cent. more extensible than equatorial strips of sclera. Weber (1877) found a large variation in the extensibility of strips of sclera from different human eyes, but gave no figures for the cornea.

Ischreyt (1899), using cattle sclera, measured the weight required to break strips of sclera, the extension of the strips at the moment of breaking, and the extension produced by increasing weights. The weights used, however, produced tensions in the sclera corresponding to intra-ocular pressures far above physiological levels.

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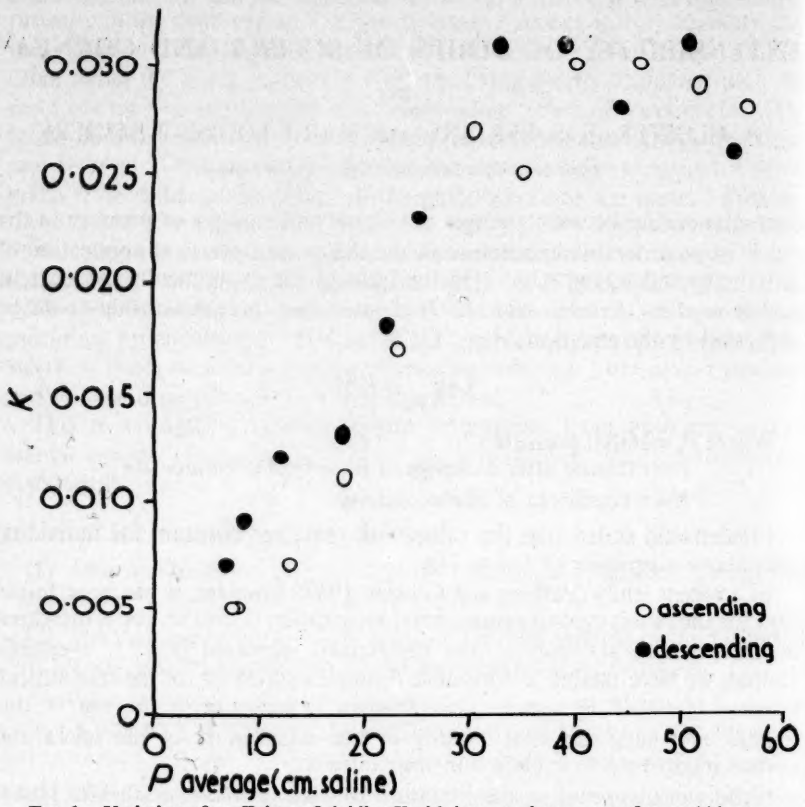

FIG. 1.—Variation of coefficient of rigidity K with intra-ocular pressure for a rabbit eye.

We have measured the extensibility of strips of sclera and cornea, using weights which produced tensions more nearly resembling those in the sclera of the normal eye.

Method

The principle of the method employed consisted in suspending strips of cornea or sclera from one arm of a balance with the lower end of the strip fixed. Weights were added to the opposite scale pan and the extension of the strip was measured by the excursion of the pointer attached to the beam.

The strips were cut from enucleated rabbit eyes by means of two razor blades clamped together with their edges 1 mm. apart. Except at the extremities where the strips were made wider to provide a larger surface area for clamping, the strips were 1 mm. wide. Both equatorial and meridional strips from the sclera were studied.

The strips were held by small perspex clamps, the upper one being suspended

from one arm of the balance while the lower clamp was fixed to a metal rod held vertically on a heavy platform standing over the balance pan (Fig. 2). The height of the rod could be adjusted and the rod could be clamped to the platform, thus forming a secure anchor for the lower end of the strip. A perspex chamber filled with saline surrounded the strip to prevent dehydration.

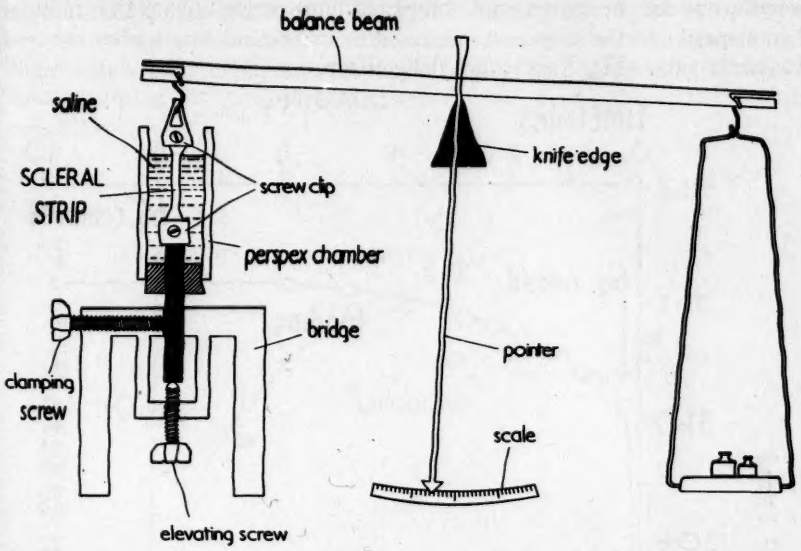

FIG. 2.—Diagram of apparatus for determining extensibility of strips of sclera and cornea.

The lower clamp attached to the metal rod was first raised until the whole weight of the strip was taken by the upper clamp. The upper clamp and strip of sclera were counterbalanced by adding weights to the right-hand scale-pan; the metal rod was then lowered until the sclera was straightened but not under tension. A 500 mg. weight was then added to the right-hand scale-pan and the movement of the pointer *P* was measured at frequent time intervals until a steady reading was obtained. Further 500-mg. weights were added until a total of either 3 or 6 g. was reached. The weights were then removed successively and a series of readings taken as before.

The pointer of the balance was fitted with an optical magnifying system allowing accurate reading to one division on the scale. Calibration by means of a micrometer attached to the point of suspension of the strip showed that a movement of the pointer of one division corresponded to a change in length of 0·015 mm.

It can be shown that the tension *T* in the strip expressed in g.wt/cm. is given by the formula:

$$T = 10\times (W - S_o + S),$$

where S_o = Initial scale reading,
S = Scale reading after adding weight,
W = Weight added in grammes.

Results

The first property of the strips of sclera and cornea which was noticed was the relatively long interval required for a steady state to be reached after the addition or removal of each weight. The greater part of the change in length took place in the first 15 seconds after the addition or removal of the weight, but the change was not completed until periods of up to 6 minutes had elapsed and the strip never returned to its original length after removal of the weights. Fig. 3 illustrates these effects.

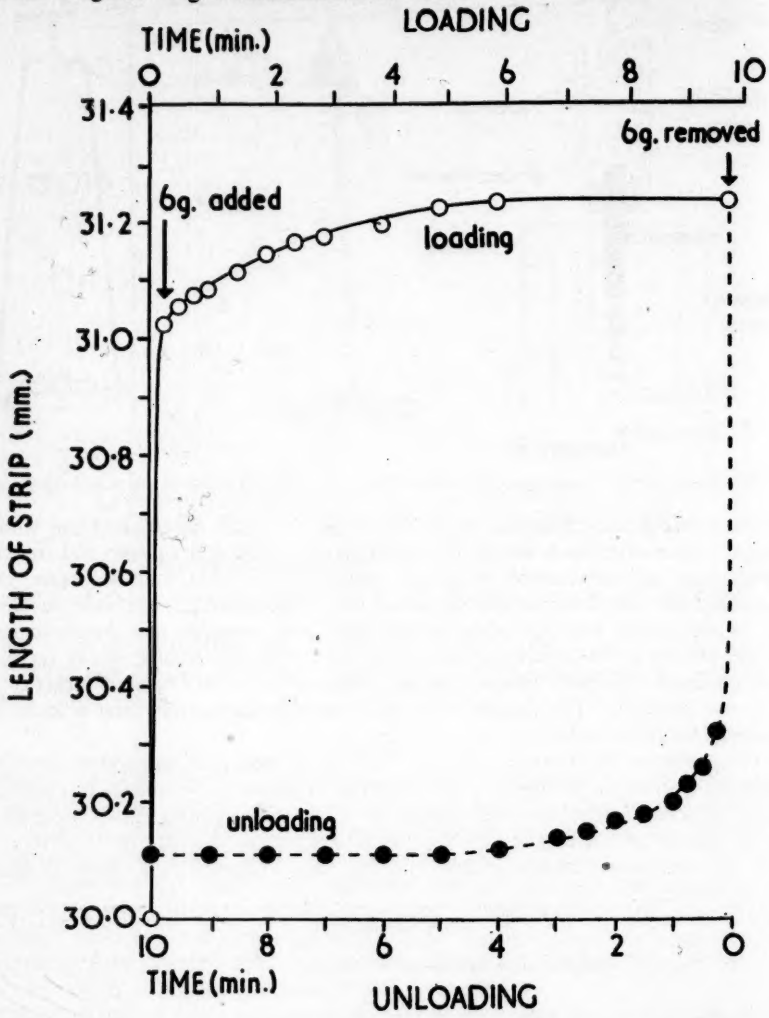

FIG. 3.—Rate of change in length of a strip of sclera after addition and removal of a 6-g. weight.

The elongation for a given increase in tension became progressively less as the total tension increased up to approximately 15 g.wt/cm., after which the elongation was nearly proportional to the increase in tension up to the maximum used—i.e. 60 g.wt/cm.

In Fig. 4 the unbroken line represents the elongation of a strip of sclera as the tension was increased. The broken line represents the behaviour of the same strip as the tension was reduced. As can be seen in this example, there were smaller changes in the length of the strip for the same changes in tension during the removal of the weights than during the addition of the weights.

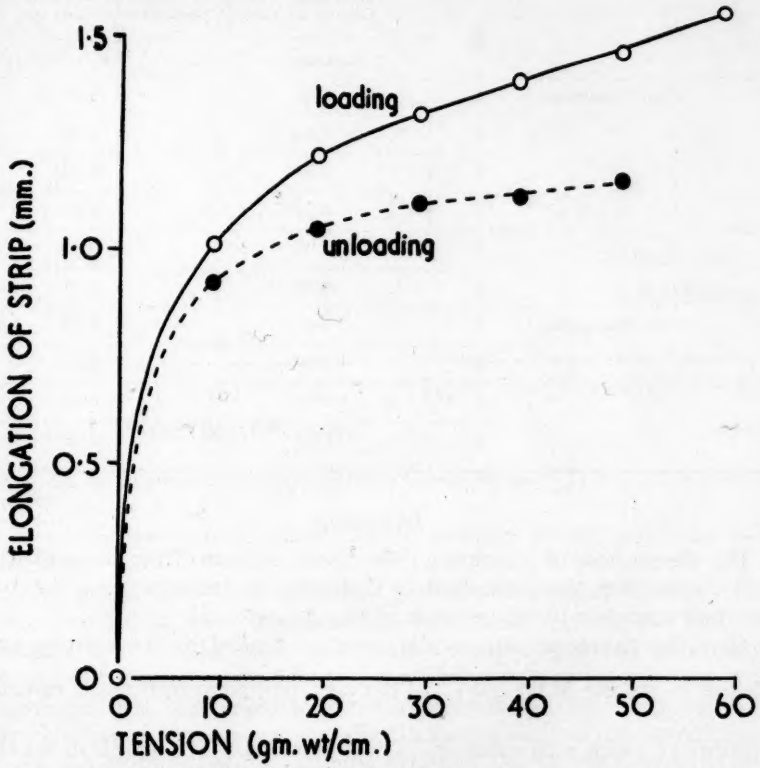

FIG. 4.—Change in length of a strip of sclera with increasing and decreasing tensions.

A convenient way of comparing strips from different eyes is to express the percentage increase in the length of the strip for an increase in tension of from 10–15 g.wt/cm. This figure was chosen as it corresponds approximately to a change in intra-ocular pressure of from 20 to 30 cm. saline. The results from eight scleral strips (six equatorial and two meridional) and two

corneal strips are shown in the Table (below). The figures show a fairly wide variation between individual eyes, the figures during loading always being higher than the figures during unloading. No significant difference was apparent between the equatorial and meridional scleral strips nor between the cornea and sclera, although the existence of such differences might be revealed in a larger series of experiments.

TABLE
EXTENSIBILITY OF STRIPS OF SCLERA AND CORNEA

Strips			Percentage Change in Length of Strips for Change in Tension from 10 to 15 g.wt/cm.	
			Loading	Unloading
Sclera	(*a*) Equatorial	1	0·37	0·23
		2	0·43	0·25
		3	0·93	0·10
		4	0·52	0·22
		5	0·94	0·38
		6	0·48	0·33
	(*b*) Meridional	1	0·80	0·52
		2	0·38	0·29
Cornea		1	0·92	0·42
		2	0·67	0·40

Discussion

For the purpose of comparing these results obtained from strips of sclera and cornea with those obtained by distension of the intact eye, the latter are most conveniently expressed as in Fig. 5 (opposite).

Here the change in intra-ocular pressure divided by the corresponding change in volume of the eye $\left(\frac{\Delta P}{\Delta V}\right)$ has been plotted against the intra-ocular pressure at which each value of $\frac{\Delta P}{\Delta V}$ was obtained. This graph shows that, as the intra-ocular pressure rises, the increase in intra-ocular pressure induced by the same change in volume of the eye becomes greater. This means that the eye becomes less distensible at higher pressures, and, if this is due to the physical properties of the sclera, one would expect isolated strips of sclera to become less extensible as the total tension in them is increased. Our results agree with this expectation for tensions up to

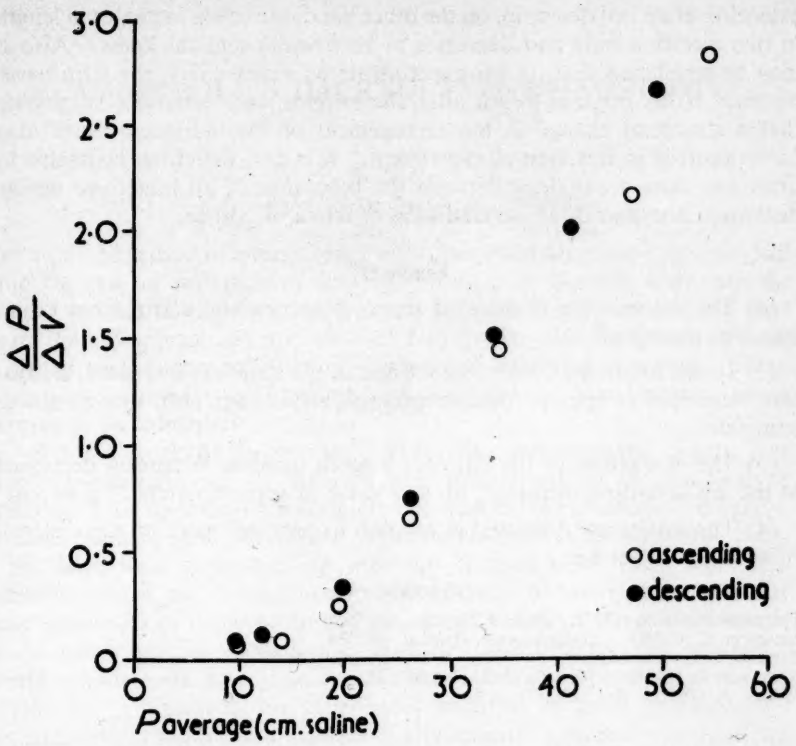

FIG. 5.—Change in pressure per unit change in volume in the eye $\left(\frac{\Delta P}{\Delta V}\right)$ at various intra-ocular pressures.

20 g.wt./cm., but above this level the extensibility tended to become more constant.

In the experiments on distension of the eye (Perkins and Gloster, 1957), it was found that, when measurements were made after the eye had been subjected to a raised intra-ocular pressure (solid circles in Fig. 5), larger changes of pressure resulted from a constant change of volume than in the undistended eye. In the present series of experiments, the elongation for a given change of tension was less during the removal of the weights than during the addition of the weights (Table). As in the previous comparison, there is some measure of agreement between the results of the two types of experiment.

There are certain factors which make it unlikely that the two sets of data would agree in greater detail. For example, during distension of an intact eye, the cornea and sclera can be considered to stretch in two directions at right angles to one another and to decrease in thickness. During the

extension of an isolated strip, on the other hand, the sclera increases in length in one direction only and decreases in both width and thickness. Also it may be significant that, in the experiments on extensibility, the strip never returned to its original length after the weights were removed, suggesting that a structural change in the arrangement of the individual fibres may have occurred in this type of experiment. It is not, therefore, justifiable to draw too close an analogy between the behaviour of an intact eye during distension and that of an isolated strip of sclera or cornea.

Summary

(1) The extensibility of isolated strips of cornea and sclera from rabbit eyes was measured.

(2) It was found that, when the tension in the strip was increased, elongation was rapid at first but became progressively slower until stretching was completed.

(3) The elongation of the strip for a given increase in tension decreased as the initial tension increased, up to a value of approximately 15 g.wt./cm.

(4) The results are discussed in relation to previous work on the distensibility of the intact eye.

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SUMMERSKILL'S DACRYOCYSTORHINOSTOMY*

BY

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THE routine excision of lacrimal sacs with obstructed drainage is bad surgical practice, and the provision of drainage through the lacrimal bone into the middle meatus of the nose has become the accepted procedure. In past years the time-consuming operation of Toti and its modifications have been accepted as the only satisfactory methods of achieving this aim. These operations, however, are tedious and troublesome, and require much experience for consistent success.

But the work of Summerskill (1952) has demonstrated clearly that excellent drainage can be achieved and maintained by the simplest of methods. His operation has certainly not received the attention it deserves, for it represents a great advance on any other previously published procedure.

We have been experimenting with this method since 1953, and have achieved almost consistently good results. Since it has presented for our busy eye-clinics so many advantages in speed, simplicity, and effectiveness, we decided to publish a small series of cases in the hope that this procedure would win far more general acceptance than it has done previously.

This report represents the experiences obtained by using Summerskill's method, slightly modified, in a series of 36 consecutive cases of naso-lacrimal obstruction operated on at our annual eye-clinics in Shikarpur and Khairpur, Pakistan, in January, February, and March, 1955.

Material

(*a*) *Cases with lacrimation and a muco-purulent discharge on pressing over the inner canthus.*—The duration of symptoms was several months to 2 or 3 years. The ages of the patients ranged from 2 or 3 to 50 or 60 years. Nearly half the patients were children between 3 and 4 years old, and the large majority of the patients were female. (The ages of patients here have to be assessed by guesswork since many of the poorer class have no idea of their age.) This group comprised 29 patients.

(*b*) *Cases with fistulae which had been discharging mucus or muco-pus for months or years.*—These were all found to have excess fibrous tissue around the sac, together with some overlying oedema due to recent inflammation. This group comprised 7 patients.

In one such case the superficial and deep tissues were red, swollen, and acutely inflamed; after a few injections of procaine penicillin 400,000 units per day, the

* Received for publication October 25, 1956.

redness disappeared, but the pus from the fistula and the oedema and friability of tissues remained. Nevertheless, the operation was performed owing to pressure from the patient who was poor and could not afford to stay away from his work longer. The result was perfect and healing by first intention occurred without any trouble to the patient or to the nursing staff.

Some patients presented with symptoms of mucoid discharge onto the face arising from sacs which had previously been operated on here or elsewhere by other methods. At operation some sacs were found intact and had obviously not been found at the first operation. Some had previously been operated on by the Summerskill method by beginners in our clinic. In these the polythene tubing had evidently not been accurately pushed home, and lay loosely in the hole made for it. In such cases it was found on opening the sac that the tube was lying transversely in a distended sac cavity. There was no internal fistula into the nose and the tears and the mucus were being discharged on to the face; there was no tissue reaction around the tube, but it was simply not fulfilling its purpose. All that was required in these cases was to excise the skin edges, create a fresh internal fistula into the middle meatus of the nose, insert a fresh tube so that it fitted snugly, and sew up the skin.

This group included two patients who presented cystic dilatations of the sac which were found to be recurrences due to a previous Summerskill's operation incorrectly done. The mucoid discharge drained backwards through the punctum, but no fistula was present. These were very easy to deal with. On incising the skin, a clean dilated sac filled with mucus was found, with the polythene tube lying transversely in it. The tube was removed, a new fistula was established into the nose, and a fresh tube was pushed home.

Summerskill (1949) gave reasons for abandoning intubation of the naso-lacrimal duct in favour of this simpler and more effective procedure, later more fully described by him (1952). Our method follows the later technique in all essentials, except that the opening into the nose is made by a trocar and cannula and that the only sutures we insert are those into the skin.

Operative Procedure

The nasal cavity on the appropriate side is packed with gauze impregnated with a solution of Nupercaine H.Cl. 1 per cent with adrenaline 1/1,000 in equal parts 10 minutes before operation. Under local infiltration anaesthesia combined with a block of the naso-lacrimal nerve in the medial wall of the orbit, a vertical incision is made about 3 mm. medial to the inner canthus. The skin and orbicularis muscle are retracted. Operations in children are done under intra-tracheal anaesthesia. The tissues around the sac are first infiltrated with a few mls. 1/2,000 Nupercaine-adrenaline solution in order to achieve haemostasis. (This is done after the patient is anaesthetized and some 4 or 5 minutes before the operation is begun.) The lacrimal fascia is incised and dissected off the sac which is then opened and its mucoid or muco-purulent contents swabbed out. A trocar and cannula of suitable size is passed through the lower end of the medial wall of the sac and on through the lacrimal bone into the nasal cavity in one motion. The instrument is then removed, and the polythene tube, mounted on a simple introducer, is passed through the hole so that the lip of the tube rests firmly against the medial wall

of the sac. The tube must fit snugly and the introducer should fit loosely into the tube so that it does not pull the tube out with it on withdrawal. The cause of failure in this operation has sometimes been that the introducer was fitted too tightly into the tube, so that the tube was partially withdrawn when the introducer was removed; thus, in course of time, the tube comes to lie in the cavity of the sac and the stoma closes.

The position of the tube in the sac is always verified by inspection and the patency of the fistula into the middle meatus of the nose is tested by inserting a probe through it. The sac itself is not stitched, but the skin edges are accurately sewn together with interrupted cotton sutures. A firm pad and bandage are applied.

The dressing is untouched for 48 hours and is subsequently changed on alternate days. The stitches are removed on the sixth day after operation.

Commentary

Healing of Wounds.—Over 90 per cent of the cases in this series healed by first intention. A few wounds, especially those in sacs with previous fistulae, were treated with hot fomentations three or four times a day for 4 or 5 days. With this procedure healing soon occurred. Two patients with fistulae needed between 10 and 15 days to heal, but all others cleared up within 10 days.

Speed of Operation.—In place of a long drawn-out procedure the whole operation can be completed with a speed which will astonish the operator himself. Once the simple steps in identifying and opening the sac are mastered (and this, surely, is familiar to anyone who excises sacs routinely), the insertion of the tube and the stitching of the wound can be done very quickly. As soon as the anaesthesia is established and the field prepared it has been found that the total time taken for the operation in 80 per cent of cases varies from 3 to 5 minutes. This fact alone commends itself to us in Pakistan where we have had to perform an average of over forty cataract extractions and over twenty other major ophthalmic operations daily for 2 weeks in one of our clinics in 1955.

Simplicity.—The description of the operation has only to be verified in practice to demonstrate how simple it is.

Effectiveness.—The early results were most pleasing to patient and operator alike. The symptoms were immediately relieved and the wounds healed quickly. It is too early to comment on the long-term results of the operation except to say that of more than one hundred cases of lacrimal obstruction operated on since 1953, we have seen only about a dozen patients presenting with a recurrence of symptoms, and all but one of these had been operated on by beginners. The mishaps were all caused by the displacement of the polythene tube, and all were successfully corrected by a subsequent operation.

Post-Operative Discomfort.—The patients themselves were impressed by the little discomfort they suffered. This was hardly noticeable in over

90 per cent of patients; a few who had surrounding inflammation or fibrosis before operation complained of slight pain, but even these rarely needed sedatives.

Summary

(1) A series of 36 cases of lacrimal obstruction is presented.

(2) All were operated on by a modification of Summerskill's method of dacryocystorhinostomy by means of a polythene tube.

(3) Cases of simple obstruction and cases presenting fistulae, with or without overlying inflammation, were all successfully treated by this method.

(4) It is claimed that Summerskill's operation represents a definite advance over other methods, and the procedure is recommended for all types of obstruction of the naso-lacrimal duct. A plea is made for its wider acceptance in view of its advantages of speed, simplicity, effectiveness, and absence of post-operative discomfort.

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Brit. J. Ophthal. (1957) **41**, 115.

AIR IN THE EYE*

BY

A. LISTER

London

THE introduction of air, or saline, into the eye is a fairly recent innovation. The practice has its advocates and its critics, so that a short review of its uses by one of the former may be of interest. The author's experience, chiefly with air, forms the basis of this paper. Recently, however, it has become apparent to him that there are occasions when saline is preferable. In the conclusion, therefore, the relative merits of air and saline are briefly discussed.

In the anterior chamber, the most common use of air is probably after cataract extraction. It has even been stated that the injection of air is the most important part of the operation! In most instances it is unnecessary because, with the use of sutures—which again is a controversial matter—the anterior chamber reforms rapidly. There are, however, occasions when the iris is lacking in tone and when at the end of the operation it seems wise to push it away from the section with a bubble of air. This applies to both extra- and intra-capsular extractions.

The only likely serious complication is the passage of the air through the pupil, behind the iris, where of course it does the opposite of what is desired. The writer was first made aware of this possibility in a patient in whom a large bubble of air entered in the "wash-out" and filled the anterior chamber. All looked so satisfactory that it was decided to leave it there and the patient went back to bed in Fowler's position. Next day the iris was found to be hard up against the section with no sign of any air. It seemed advisable to abscise the threatened prolapse and the patient came back to the theatre for this to be done. As she lay flat on the table a bubble of air emerged through her pupil into the anterior chamber. Even then what had really happened was not understood and the abscission was completed. It was only later, when a rather similar event occurred in another patient, that the problem was solved.

This danger is sufficient to antagonize some surgeons against the use of air after a cataract operation, but it is quite easily avoided by ensuring that the patient is kept reasonably flat—with not more than three pillows instead of a bed-rest for the first day or two after operation.

Air is probably contraindicated where there has been a complete

* Received for publication, November 9, 1956.

iridectomy, and certainly where there is vitreous loss. On one occasion when there was vitreous loss after an attempted linear extraction of a congenital cataract the only effect of the air was to push more vitreous out.

Air can be used only if the section is closed with sutures and there are many who consider sutures and, still more, air to be unnecessary complications of the cataract operation. The answer, as far as the writer is concerned, is that they have made iris prolapse an extreme rarity and have allowed us to take liberties with elderly patients—such as leaving the pupil intact and getting them up the day after operation—which would have been a serious risk in pre-suture days.

Another operation in which air is useful on occasions is penetrating keratoplasty. As is well-known there is seldom a risk of an anterior synechia provided that during the operation the pupil border is either inside or outside the edge of the graft, so that for grafts of 5 mm. one likes to operate with a large pupil, and for grafts of 6 mm. or more one operates with the pupil contracted by miotics. There are, however, some pupils which, in spite of intensive pre-operative miotics, dilate under the influence of pantocaine and the retrobulbar anaesthetic, usually to about the same size as the graft. In such cases, and in those in which the pillars of an iridectomy cross the edge of the graft, it is an advantage to make sure that the iris is clear of the graft at the end of the operation with a few bubbles of air. If one uses direct sutures there is no risk of the graft being displaced by the air. The air may be introduced with a No. 20 needle with the point filed off, inserted along the track of a small paracentesis puncture made early in the operation—usually just after marking the recipient cornea with the trephine.

After total keratoplasty, too, one may follow Castroviejo's instructions and fill the anterior chamber with air, so as to keep the vitreous away from the graft.

Stallard (1955) recommends that, in graft operations where the anterior chamber is shallow or has been lost through a too deeply inserted "preplaced" suture, it may be deepened or restored so as to make trephining the cornea safe by means of air injected through a cyclodialysis incision.

A third operation which may be completed with advantage with the injection of air into the anterior chamber is cyclodialysis, as advocated by Rome and Koff (1948). The air, besides hastening the re-formation of the anterior chamber and thus, one hopes, keeping the cyclodialysis cleft open, stops haemorrhage if there is any. It acts, as the authors quoted put it, like an "internal tourniquet". The air is introduced through the cyclodialysis opening with a fine lacrimal cannula. After goniotomy too, which is similar in a way to cyclodialysis, reformation of the anterior chamber immediately after the operation does at least theoretically help to prevent the re-formation of goniosynechiae. Incidentally, it is sometimes possible to reform the anterior chamber with air after goniotomy by merely "squirting" the air at the site of entry of the goniotomy needle.

Some years ago it was suggested that goniotomy could be facilitated by replacing the aqueous with air, thus giving a view of the angle without having to use a gonioscopic lens. Unfortunately the contact of the bubble with the posterior surface of the cornea makes a practically opaque screen through which details of the angle are invisible.

Another occasion in which air is of great value—perhaps its greatest—is in the repair of a perforated eye, when it is often extremely difficult to ensure that uveal tissue, lens capsule, or vitreous is clear of the wound. This point is best illustrated by a case report.

A small boy was cutting a piece of string with a nail file when the file slipped and perforated the eye, making a jagged wound involving both cornea and sclera. The lens was badly damaged and there was prolapse of lens and iris from the wound. At operation the prolapse was abscised as effectively as possible and the lens capsule was drawn out of the wound followed by a "bead" of vitreous. The wound was then closed with direct sutures.

Clearly it was quite impossible to free the wound entirely of intra-ocular tissue, and one was faced with the prospect of extensive anterior synechiae and an exceedingly dangerous eye. Luckily it was possible to restore most of the anterior chamber by injecting air through the wound. Ten days later there was still a small band of capsular synechia. This was divided, but in doing so the wound opened slightly and the anterior chamber was lost. However, it was this time completely reformed by an injection of air through a paracentesis puncture. The eye settled down well and though it has grossly defective sight is at least a cosmetic asset. It would certainly have been lost without the use of air.

This case illustrates the important place that air has in the treatment of penetrating wounds and, incidentally, of anterior synechiae. In the latter instance, the chances of recurrence are greatly lessened if, after the synechia has been divided, the anterior chamber is re-formed with air injected through a small "pre-placed" paracentesis puncture.

One might add that the use of air has largely been made possible by the exceedingly fine suture materials—needles and silk—which are available to us nowadays. These have, in the writer's view, rung the knell of the conjunctival flap—never a satisfactory method of closing a penetrating wound.

Lastly, as far as the anterior chamber is concerned, is the use of air in restoring the chamber when its re-formation is delayed after a filtering operation or after cataract extraction. The writer has been fortunate in having little experience of this complication in the latter, but Kronfeld (1954) speaks of it as being fairly common and advocates the injection of air into the anterior chamber combined with drainage of the choroidal detachment which is a frequent complication.

The writer's experience with filtering operations has been less happy, and to him delay or failure of re-formation of the anterior chamber after a trephine has, in elderly patients, been a discouragingly common complication. The answer probably lies in prevention by modification of technique. However that may be, in the few cases in which restoration of the anterior chamber with air has been attempted it has never lasted more than a day or two.

In the posterior chamber the use of air in the treatment of retinal detachment is becoming increasingly common. It may be illustrated by the following three cases:

An elderly stout woman with an upper quadrant detachment would have found the normal posture, on her back, quite impossible. She was therefore nursed in Fowler's position, and the large bubble of air which was injected into the vitreous at the end of the diathermy operation kept her retina in place and it became firmly re-attached.

An elderly man, who also had a detachment with an upper quadrant tear, found it quite impossible to keep still in bed lying on his side, and a week after operation, although the retinal hole appeared to be covered by diathermy, there was a large temporal balloon which seemed to be increasing. Without further diathermy, the balloon was tapped and air injected into the vitreous, after which the patient was nursed in Fowler's position, with a most satisfactory result.

A boy aged 12 had a total detachment in which no hole could be found. A full circle lamellar scleral resection was performed in two stages, air being injected at the end of each operation. After each the retina was entirely replaced for a short period but subsequently redetached. A third attempt, combining a diathermy barrage with air injection, again replaced the retina, this time permanently. This case shows that by means of air a total detachment may be so far replaced as to give it some chance of adhesion where adhesion is necessary, thus improving the prognosis in a type of case which has generally been considered hopeless.

These three cases illustrate two obvious ways in which air may be of assistance, namely in overcoming gravity in the elderly patient and in "controlling" a totally detached retina. It has a third possible action which is worth mentioning; that is of breaking down adhesions between vitreous and retina which would prevent the replacement of the latter.

The effect of an air injection upon the vitreous itself is not known. Presumably it is not beneficial, although in most cases the vitreous is already degenerate. There is no question therefore of advocating air injection in more than a minority of cases of retinal detachment, and it is, in fact, contra-indicated when there is a particularly large tear owing to the risk of the air passing behind the retina. In aphakia there is a risk of the air coming forward into the anterior chamber. This might not matter but it can be prevented by suitable positioning of the patient.

Redmond Smith (1952) reported success in three out of five difficult cases in which air was injected into the suprachoroidal space instead of into the vitreous.

Technique

The technique of air injection is simple. Sterility may be achieved either by autoclaving already loaded syringes or by drawing up the air through a flame or through sterile cotton wool.

A No. 20 needle with the point filed off is satisfactory for injecting air into the anterior chamber through the wound of either operation or injury or through a pre-placed paracentesis puncture. In cyclodialysis a lacrimal needle is preferable because its smooth tip is less likely to penetrate or injure the ciliary body. In

retinal detachment the injection is made into the vitreous, after the subretinal fluid has been tapped, with a sharp No. 20 needle inserted 8 mm. from the limbus —i.e. through the region of the pars plana of the ciliary body. It facilitates the entry of the needle to make a small preliminary incision half or three-quarter way through the sclera, and the eye may be fixed by grasping one edge of this incision with fine-toothed forceps. Otherwise piercing a soft eye is apt to be difficult. Another aid is to have a small "stop" of solder on the shaft of the needle about 3 mm. from its point. This prevents the needle going in too far.

Choice between Air and Saline.—In retinal detachments, particularly those in the elderly when the tear is in the upper part of the eye, air seems to be the obvious choice, though this is not the view of everyone. In the anterior chamber, air has the advantage that owing to its greater surface tension it is less likely to leak than saline. This surface tension may, however, sometimes be a disadvantage, preventing the air from spreading where one wants it. For example, in a corneal graft, a bubble of air, in spite of efforts to dislodge it, remained localized in one part of the anterior chamber, forcing the iris elsewhere against the back of the graft. It was therefore withdrawn and saline introduced in its place, with good results. A further point is, that being more foreign to the eye than normal saline, air may be more irritating.

Opinions differ among different people and vary from time to time in the same person. That of the writer at the present time is that saline is to be preferred when it fulfils the desired purpose.

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Postscript

Since this article was written the author has encountered a complication of which he had heard but had not experienced, namely air-lock of the pupil. It occurred in a case in which air had been introduced after a cyclodialysis. On the following day the pupil was found to be sealed by the air bubble with consequent iris bombé and raised tension. The situation was relieved by paracentesis.

This accident was obviously possible in all cases where the iris is intact and provides a further point in favour of saline. It is probable, however, that it may be avoided by limiting the amount of air introduced and by positioning the patient so that the air does not completely cover the pupil.

Brit. J. Ophthal. (1957) **41**, 120.

CASE NOTES

CONGENITAL IRIS LESION*

BY

W. J. LEVY

St. Thomas's Hospital, London

THE congenital iris lesion reported below is probably unique, and is for this reason recorded as an interesting case.

Case Report

A student teacher of Turkish-Cypriot origin, whilst in Great Britain on a British Council visit, inquired whether anything could be done to alleviate an eye condition which had troubled him since birth. He had previously been told that nothing could be done for it.

He complained of poor vision in both eyes since birth, the left being worse than the right. He had had glasses prescribed but they were no longer of any assistance to him. There was no family history of any eye trouble, nor were his parents consanguinous He was born of a normal, full-term pregnancy.

Examination.—Visual acuity in the right eye was 6/18pt, unaided; and in the left 6/36, unaided. No improvement in vision could be effected with glasses. Both eyes were white and quiet. The anterior chambers were of normal depth except in the immediate pupillary area. From the anterior aspect of the iris in either eye, bulged forward an almost normally coloured and slightly abnormally patterned iris tissue, commencing approximately 2·5 mm. from the pupillary margin (Fig. 1).

FIG. 1.—Schematic diagram to illustrate the billowing forwards of the mesodermal anomaly, and approximate site of origin. Anterior limits of anomaly exaggerated for the sake of clarity.

* Received for publication, September 9, 1956.

In the centre of this tissue was a pseudo-pupil, occluded by an irregular, partly opaque membrane (Figs 2 and 3).

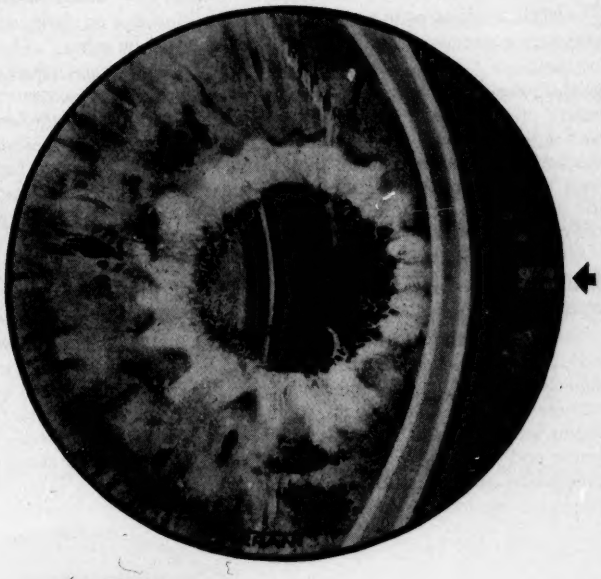

FIG. 2.—Right eye, showing well slightly transparent pupillary membrane, highlighted by the irregular slit beam, separated by aqueous from the lens, highlighted by the regular slit beam in the pupil.

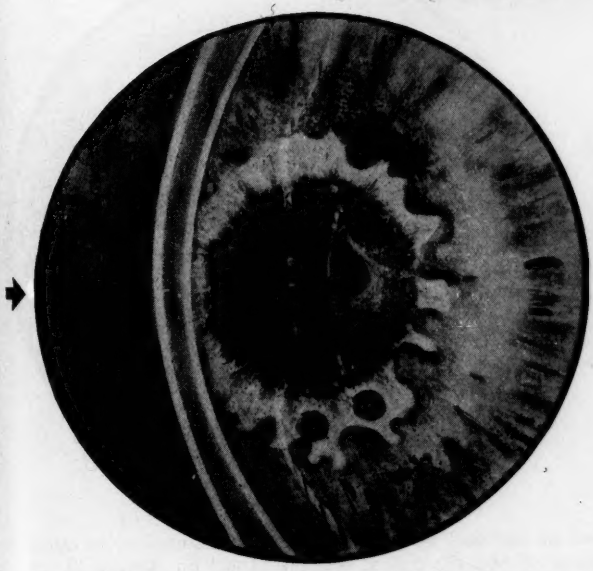

FIG. 3.—Left eye, showing a less clear pupillary membrane. The billowing forwards of the pigmented part of the mesodermal anomaly is better demonstrated here.

Through this membrane and approximately 0·5 mm. deep to it, was seen the lens (Figs 2 and 3), on which lay a normally-reacting pupillary margin and iris. Miosis caused this

pseudocyst-like lesion to become more pronounced (a fact which was utilized later at operation). In the right eye the lens could be seen to be clear, except for a small congenital sutural cataract. There was a limited view of an apparently normal fundus. The left lens appeared to be in the correct position, but its clarity could not be established, and only a minimal red reflex of the fundus could be seen.

Operation Left Eye.—On July 25, 1956, the pupil was strongly miosed and, under general anaesthetic, a superior keratome incision was made. The membrane in the centre of the pouting lesion was grasped with intra-capsular forceps. The membrane was separated from the surrounding persistent pigmented mesoderm; however, as this reached the superior margin, the atrophic iris and pigmented mesoderm remains came with the membrane and a complete, superior iridectomy resulted. The poorly elastic iris pillars were reposited with some difficulty and, once the iris was free of the wound, the clear, immediately adjacent, lens was respected (Fig. 4).

First Operation Right Eye.—On August 1, 1956, the pupil was strongly miosed. Under local anaesthetic a corepraxia was attempted with two needles. This separated the membrane of the central area along its inferior margin, but unfortunately it flapped back.

Second Operation Right Eye.—On August 9, 1956, the pupil was once again miosed and, once more under general anaesthetic, a superior keratome incision was made. The slightly anteriorly curled, inferior edge of the central membrane was grasped with intra-capsular forceps. Once again, when the superior margin of the pigmented mesodermal lesion was reached, it and the underlying atrophic iris came away with the central membrane and a complete superior iridectomy resulted. The same difficulty with the iris pillars was met. A clear lens was visible (Fig. 4).

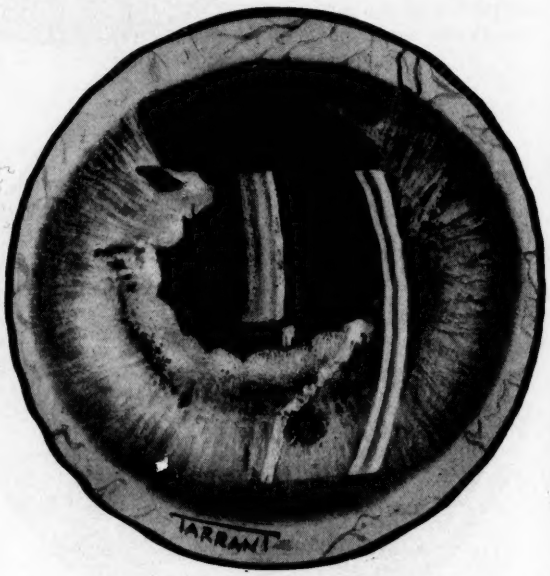

FIG. 4.—Post-operative view. The picture was the same in each eye. The remaining pigmented portion of the anomaly, billowing away from the pupil, is clearly seen. The pupil is quite clear with a clear lens.

Result.—Post-operative convalescence was uneventful and was maintained on atropine and topical hydrocortisone. When the patient was discharged on August 30, 1956, when he was returning to Cyprus, his condition was as follows:

Visual acuity: In the right eye 6/9 pt, with −1 D sph., +0·75 D cyl., axis 30°.
In the left eye 6/18 + 2, with −1·5 D sph., +1·25 D cyl., axis 170°.
Binocular vision 6/9–3.

Both eyes were white and quiet. In each eye there was a complete superior iridectomy with an anterior synechia of the temporal pillar. The pupil was quite clear and the inferior remains of the anterior mesodermal anomaly billowed forward. The lens was clear except for the congenital sutural cataract, identical in each eye. No abnormality of the vitreous or fundus could be seen in either eye.

Discussion

In this case, the pupillary membrane, which normally regresses entirely during gestation, persisted, and in association with this, a reduplicated anterior layer of the pigmented mesoderm developed. It is interesting that the pseudo-pupil should have the same limits as the normal, underlying pupil, and also that the pigmented portion of the anomaly had no muscular activity, whilst the underlying iris, although atrophic, showed normal activity.

Summary

An unusual case of iris and pupillary anomaly is reported.

I should like to thank Mr. Harold Ridley for his encouragement and advice, and for permission to publish this case record. I should also like to thank Messrs. Tarrant and Jeffreys of the Medical Illustration Department of the Institute of Ophthalmology for the paintings and photographs.

Brit. J. Ophthal. (1957) **41**, 124.

CASE NOTES

LEECH-BITE OF THE CORNEA

BY

M. GILKES

Jerusalem, Jordan

THE following case of leech-bite of the cornea is thought to be of interest, in that a survey of the available literature reveals no similar case.

Case Report

An Arab woman aged 25 attended the eye clinic during a routine visit to an UNNRA Palestine refugee camp in the hills of Judaea, to the north of Jerusalem, in September, 1956. She complained of severe pain in the right eye since washing her face in her cupped hands, with water from a nearby hillside spring, early that morning.

Examination.—A brown fleshy mass was observed lying eccentric on the right cornea, very closely simulating a prolapse of uveal tissue. Closer examination revealed this to be a leech (*'Alaqat*), with its sucker adherent to the cornea and its mouth to the limbus (*Figure*). The eye was markedly injected and obviously painful.

FIGURE.—Right eye showing leech adherent to cornea and limbus.
Marked trachomatous vessels are present in the upper cornea.

Attempts had been made to remove the leech with forceps, and the instillation of 1 per cent. pantocaine had no effect on the parasite. However, irrigation of the eye with hypertonic saline (approx. 5 per cent.) resulted in the immediate separation of the parasite, which, although reduced in size, did not appear to regurgitate any fluid.

* Received for publication, October 20, 1956.

Two large staining areas were present on the cornea and accordingly the eye was padded after the application of chloramphenicol ointment.

Subsequent progress was uneventful. When the patient was examined 3 days later the epithelium was intact and the only residuum was a faint nebula.

Discussion

Although the occurrence of leech-bites of the eyes may be unremarkable in tropical countries under jungle conditions associated with heat and moisture, (Duke-Elder, 1952), the presence of these parasites in the barren and arid hills of Judaea comes as somewhat of a surprise. Moreover, Manson-Bahr (1945) reports the occurrence of the horse leech (*Limnatus nilotica*) in springs and wells in the south of Europe and in the Middle East. A tentative identification of the specimen found in this case with this species has in fact been made. A survey of hillside springs in the area used by the population disclosed numerous other specimens in the crevices of the rock and it appears that cases of infestation of the mouth and throat of the many people who use these sources of water, owing to the sometimes severe shortage of other supplies, are not uncommon.

However the present case appeared to have been the first of its type noted in this area and possibly in Jordan, as the records of the Ophthalmic Hospital in Jerusalem disclose nothing similar.

It is tempting to speculate on the stimulus which caused the parasite to retain its hold on an avascular area, and to wonder what might be the effect of using leeches in corneal disease where oedematous changes are present.

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Brit. J. Ophthal. (1957) **41**, 126.

CORRESPONDENCE

To the Editorial Committee of the BRITISH JOURNAL OF OPHTHALMOLOGY

SIRS—In our paper "Stereopsis and Depth Perception following Treatment for Convergent Squint" (*British Journal of Ophthalmology*, 40, 641), a reference appears to the "Oxford Congress of the Faculty of Ophthalmologists". This unfortunate mis-statement, which should have read "Oxford Ophthalmological Congress", did not appear in our original manuscript and we wish to apologise both to the Faculty of Ophthalmologists and to the Oxford Ophthalmological Congress for any inconvenience that it may have caused.

Yours faithfully,
E. J. NAYLOR,
T. E. SHANNON,
A. STANWORTH.

DEPARTMENT OF OPHTHALMOLOGY,
ROYAL EYE HOSPITAL,
OXFORD ROAD, MANCHESTER, 13.
December 10, 1956.

BOOK REVIEW

Keratoplasty. By R. Townley Paton. 1955. Pp. 280, 87 figs, bibliography. McGraw-Hill, London. (£10 14*s.* 0*d.*)

Dr. Paton sets the stage for his work by a comprehensive historical survey of corneal grafting. Two brief chapters on anatomy and physiology are followed by a long and informative review of case selection where many problems are presented, each being approached in a similar manner: first the literature is reviewed and the opinions of authorities are described, and then, where he considers it to be necessary, the author states his own views in the light of his wide experience.

The problems of donor material, its selection and preservation, and the differing legal aspects of its collection in various countries are next considered. Pre-operative preparation, operative technique, and post-operative care are discussed in a section which is clearly written and beautifully illustrated by drawings. The author describes not only the operations which he himself prefers but also those of other surgeons, the advantages, and disadvantages of which are clearly indicated.

The concluding chapters deal with radiation therapy, the use of cortisone, and the histology of the healing cornea, followed by a statistical survey. An appendix contains the record of some 365 personal cases of corneal grafting—a section that possibly some will find to be the most fascinating in the book.

It is refreshing to read this book which represents the philosophy of one man—an expert in his subject. There are few omissions—the chapters on anatomy and physiology are very short and some of the views expressed are not in accord with present physiological thought. Possibly the greatest criticism is that of the high price some part of which must be due to the inclusion of 29 beautiful colour photographs of pre- and post-operative conditions. These must be as great a source of satisfaction to the author as they are of delight to the reader, but it is questionable if the value of the book would have been

affected if these colour pictures had been reproduced in black and white to accompany the many other excellent photographs in this medium.

Bibliographies are given at the end of each chapter and although these are selective and adequate they are by no means comprehensive. Whatever may be these minor criticisms, the book has fully attained the expectations anticipated from a knowledge of the stature and achievements of the author. It represents a lucid, balanced, and informative exposition of the problem of keratoplasty. Dr. Paton and his publishers are to be congratulated upon this beautifully-produced work.

BOOKS REVIEWED IN *Ophthalmic Literature*

The Blind in the Life of the Nations (in German). By M. Schöffler. 1956. Pp. 280, 14 figs, 86 refs. Urania-Verlag, Leipzig.

NOTES

STANDARDIZATION OF TONOMETERS

In view of some doubt expressed in requests for advice reaching the Tonometer Testing Station of the W.H. Ross Foundation (Scotland), it is desired to draw attention to the following points:

(1) The internationally accepted specification for tonometers refers only to the original (weighted) model.

(2) Standardized tonometers are now on the market, but it should be remembered that the latest conversion chart gives readings appreciably lower (in mm. Hg) than those to which doctors have previously been accustomed.

(3) Experimental work on the recalibration of weighted tonometers is now going on, but there is no means of recalibrating an X-tonometer.

(4) There is no standard of construction for the X-tonometer, but it is hoped that an agreed standard will be arrived at in due course. In view of the popularity of this type of instrument in the United Kingdom, efforts are being made to find a solution to the many problems involved.

XVIII INTERNATIONAL OPHTHALMOLOGICAL CONGRESS

Brussels, 1958

The first International Ophthalmological Congress was held in Brussels in 1857. To mark this centenary, the next International Congress will also be held in Brussels from 8 to 12 September, 1958.

Professor Léon Coppez will preside, and all ophthalmologists are cordially invited. It is hoped that a great number will come to Belgium, where a great Universal International Exhibition will also be held in 1958.

The main subjects chosen by the International Council are:

(1) Orthoptic Treatment of Concomitant Strabismus: presented by Dr. A. Bangerter (*St. Gallen*), Mr. T. Keith Lyle (*London*), and Professor J. Malbran (*Buenos Aires*).

(2) Geriatrics in Ophthalmology: presented by Professor Buerger (*Leipzig*), Professor J. François (*Ghent*), Professor G. Jayle (*Marseille*), and Professor M. Jayle (*Paris*).

Symposia will be organized on cataract, radio-isotopes in ophthalmology, electroretinography, and glaucoma, and other sessions will be reserved for free communications.

The International Association for the Prevention of Blindness and the International Organization against Trachoma will hold meetings during the Congress

All correspondence and requests for information should be sent to the General Secretary, Prof. Jules François, 15, Place de Smet de Naeyer, Gand, Belgium.

OBITUARY

YASUSHI NAKAMURA, 1898–1956

Yasushi Nakamura, one of the best known of Japanese ophthalmologists, died suddenly on October 18, 1956. Graduating from the Faculty of Medicine of the University of Tokyo in 1923, he was appointed professor of ophthalmology in the Nippon Medical College in 1929 where he carried out his ophthalmic practice and research until his death. His research work was well known, embracing particularly trachoma, glaucoma, keratoplasty, and embryology. His writings were profuse, and in 1947 he founded the *Journal of Clinical Ophthalmology* which has since maintained its position as one of the most important journals on the specialty published in Japan. He was the representative of the Japanese Ophthalmological Society at the XVII International Congress of Ophthalmology in North America in 1954, and played a prominent part on the Expert Committee on Trachoma held by the World Health Organization in Geneva in 1955, where he brought his profound knowledge of trachoma as it occurs in Japan to bear on the wider world problem.